CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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STATISTICAL REVIEW(S)

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/BLA:	202-811
Drug Name:	Linzess (Linaclotide) 266 μg
Indication(s):	Treatment of irritable bowel syndrome with constipation (IBS-C)
Applicant:	Forest Laboratories, Inc.
Date(s):	Received August 9, 2011 PDUFA: September 9, 2012
Review Priority:	Standard
Biometrics Division:	Division of Biometrics III
Statistical Reviewer:	Milton C. Fan, Ph.D. , DB III
Concurring Reviewers:	Mike Welch, Ph.D. , Deputy Dir., DB III
Medical Division:	Gastroenterology and Inborn Errors Products (DGIEP)
Clinical Team:	Lara Dimick-Santos, M.D., Ruyi He, M.D. (TL) (DGIEP)
Project Manager:	Brian Strongin (DGIEP)

Keywords: clinical studies, NDA review, placebo controlled

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1. EXECUTIVE SUMMARY

1.1 Conclusions and Recommendations

The sponsor has submitted two pivotal studies (MCP-103-302 and LIN-MD-31) to support the indication of treatment of irritable bowel syndrome with constipation (IBS-C). A separate statistical review addressed the chronic idiopathic constipation (CIC) indication.

Study MCP-103-302 showed that linaclotide (266 µg) was statistically significantly better than placebo in terms of the primary efficacy endpoint, 9/12 week APC 3+1 responder, based on clinical response in both abdominal pain and CSBM frequency (See Section 1.2.1 for endpoint definitions) The difference in treatment group response was 9.7%. Linaclotide was also statistically better than placebo in terms of the other three primary efficacy endpoints: 9/12 week CSBM 3+1 responder, 9/12 week abdominal pain responder, and 6/12 week APC +1 responder. The treatment differences ranged from 13% to 20%. Superiority was also shown for some secondary efficacy endpoints: change from baseline in 12-week CSBM frequency rate, change from baseline in 12-week SBM frequency rate, change from baseline in 12-week stool consistency, CSBM frequency rate, and change from baseline in 12-week percent of abdominal pain-free days.

The efficacy results from Study MCP-103-302 were replicated in Study LIN-MD-31 for the primary efficacy endpoint: 9/12 week APC 3+1 responder. However, the treatment difference was modest at 7.0%.

Per our request, the sponsor provided an analysis of the proportions of responders (abdominal pain and CSBM (APC) 3 +1) by week and by month. Greater proportions of patients in the linaclotide group were observed at almost every week and every month over the 26-week period in Study MCP-103-302. Similar results were observed during the 12 weeks for Study LIN-MD-31.

In conclusion, both studies (MCP-103-302 and LIN-MD-31) showed that linaclotide was superior to the placebo with regard to the protocol-specified endpoints.

Regarding safety, greater proportions of subjects with adverse events were observed in the linaclotide group compared with subjects in the placebo group for both studies. These comparisons include subjects with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea.

1.2. Brief Overview of Clinical Studies

1.2.1 Study MCP-103-302

This is a phase 3, randomized, double-blind, placebo-controlled, parallel-group trial of Linaclotide administered orally for 26 weeks in patients with irritable bowel syndrome with constipation (IBS-C). The trial was conducted in the U.S. (111 sites).

The objective of this trial was to determine the efficacy and safety of linaclotide administered to patients with irritable bowel syndrome with constipation (IBS-C).

Patients were randomly assigned to 1 of 2 treatment groups (linaclotide dose of 266 LIN-MD-31/day or placebo) in a 1:1 ratio. The randomization assignments were generated in blocks of 4 to facilitate balanced distribution of patient assignments across centers.

The primary efficacy parameters consisted of two components:

1) Abdominal Pain at its Worst and
2) CSBMs.

The daily patient assessments were used to determine the primary efficacy parameter.

The primary efficacy parameters assessed abdominal pain and BMs that met the criteria for CSBMs. There were 4 primary efficacy parameters:

1) 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responder: A weekly APC 3+1 Responder was a patient who had at least 3 CSBMs and an increase of at least 1 CSBM from baseline, and a decrease of at least 30% in the mean abdominal pain score, during a particular week. A 9/12 Week APC 3+1 Responder met these criteria for at least 9 of the first 12 weeks of the Treatment Period.

2) 9/12 Week CSBM 3+1 Responder: A weekly CSBM 3+1 Responder was a patient who had at least 3 CSBMs and an increase of at least 1 CSBM from baseline during a particular week. A 9/12 Week CSBM 3+1 Responder criteria met these criteria for at least 9 of the first 12 weeks of the Treatment Period

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3) 9/12 Week Abdominal Pain Responder: A weekly Abdominal Pain Responder was a patient who had a decrease of at least 30% from baseline in the mean abdominal pain score during a particular week. A 9/12 Week Abdominal Pain Responder met these criteria for at least 9 of the first 12 weeks of the Treatment Period.

4) 6/12 Week APC +1 Responder: A weekly APC +1 Responder was a patient who had an increase of at least 1 CSBM from baseline, and a decrease of at least 30% in the mean abdominal pain score, during a particular week. A 6/12 Week APC +1 Responder met these criteria for at least 6 of the first 12 weeks of the Treatment Period.

For each primary efficacy parameter, a patient had to have ≥ 4 complete IVRS calls for a particular Treatment Period week to be considered a responder for that week.

1.2.2 Study LIN-MD-31

The study design for this study was similar to that for Study MCP-103-302 with exceptions listed below. A total of 118 centers (111 in the United States, 7 in Canada) enrolled patients into the study.

This study was a phase III, randomized, double-blind, placebo-controlled, parallel-group trial of linaclotide administered orally for 12 Weeks followed by a 4-Week randomized withdrawal period in patients with irritable bowel syndrome with constipation (IBS-C).

The objective of this trial was to determine the efficacy and safety of linaclotide administered to patients with irritable bowel syndrome with constipation (IBS-C).

This study was designed for comparing a 266 LIN-MD-31/day dose of linaclotide with placebo in patients who met modified Rome II criteria for IBS-C. An interactive voice response system (IVRS) was used by study sites to randomize patients, supply study drug, and record the patient diary information.

The trial consisted of up to 21 days of screening (screening period), 14 to 21 days of pretreatment (pretreatment period), 12 weeks of double-blind treatment (treatment period), and a 4-week double-blind randomized withdrawal (RW) period.

1.3 Statistical Issues and Findings

Study MCP-103-302 showed that linaclotide was statistically significantly better than placebo in terms of the primary efficacy endpoint, 9/12 week APC 3+1 Responder. The treatment difference was 9.7%. It was also statistically better than placebo in terms of other three primary efficacy endpoints: 9/12 week CSBM 3+1 Responder, 9/12 week Abdominal Pain Responder, and 6/12 week APC +1 Responder. The treatment differences ranged from 13% to 20%. Superiority was also shown for some secondary efficacy endpoints: change from baseline in 12-week CSBM frequency rate, change from baseline in 12-week SBM frequency rate, change from baseline in 12-week stool consistency, CSBM frequency rate, and change from baseline in 12-week percent of abdominal pain-free days.

The efficacy these results from Study MCP-103-302 were replicated in Study LIN-MD-31 for primary efficacy endpoint: 9/12 week APC 3+1 Responder. However, the treatment difference was modest at 7.0%.

It was found that the sponsor failed to perform gender, age and race subgroup analyses of the proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders.

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders for gender, age, race, BMI at baseline, and abdominal pain at baseline.

Results from these subgroup analyses showed that treatment effects were consistent between studies for gender, age (<65), race, BMI at baseline ($\geq$ 30 kg/m^2) and abdominal pain (<5 and $\geq$ 5 < 8).

It was found that the sponsor failed to analyze numbers of responders for abdominal pain and CSBM (APC) 3 +1 by week and by month.

As per request, the sponsor provided analysis of the number of responder for abdominal pain and CSBM (APC) 3 +1 by week and by month. The linaclotide group showed greater proportions of responders at almost every week and every month during the 26 weeks for Study MCP-103-302. Similar results were observed during the 12 weeks for Study LIN-MD-31.

In conclusion, both studies (MCP-103-302 and LIN-MD-31) showed that linaclotide was superior to the placebo for protocol-specified endpoint, 9/12 week APC 3+1 responder. The treatment difference was 9.7% and 7.0%, respectively.

This reviewer's safety analyses showed that adverse events occurred more frequently in the linaclotide group as compared with placebo for both studies. These comparisons include the number of subjects with at least one AE, those with at least one treatment related AE (TRAE), those withdrawn due to AE, and those with at least one episode of diarrhea, and those discontinued due to TRAE of diarrhea

This reviewer notes that the lower dose of linaclotide (133 μg) was not included in these studies but was included in the studies for chronic idiopathic constipation (CIC) and results from CIC studies showed no treatment differences between low dose and high dose in one of the two pivotal studies.

2. INTRODUCTION

2.1 Overview

Linaclotide is a minimally absorbed 14-amino-acid peptide that acts locally in the intestinal lumen to stimulate the guanylate cyclase subtype C (GC-C) receptor. By activating the GC-C receptor, orally administered linaclotide has been found in animal models to increase intestinal fluid secretion and intestinal transit, and also to decrease visceral pain.

Linaclotide, a 14-amino acid synthetic peptide, is a potent and selective GC-C receptor agonist structurally related to the endogenous guanylin peptide family. Activation of the GC-C receptor results in an increase in both intracellular and extracellular concentrations of cyclic guanosine monophosphate (cGMP). Elevation in intracellular cGMP stimulates

secretions of chloride and bicarbonate into the intestinal lumen, through activation of the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel, resulting in increased intestinal fluid and accelerated transit. Extracellular cGMP decreases the activity of pain-sensing nerves, which is thought to be responsible for the observed reduction in visceral pain.

The sponsor seeks marketing approval for linaclotide as an orally administered treatment for irritable bowel syndrome with constipation (IBS-C) and chronic idiopathic constipation (CIC).

2.2 Data Sources

The sponsor has submitted two adequate and well-controlled studies (MCP-103-302) and LIN-MD-31) for the irritable bowel syndrome with constipation (IBS-C) indication and two adequate and well-controlled studies (MCP-103-303) and LIN-MD-01) for the chronic idiopathic constipation (CIC).

The four pivotal studies are listed below.

MCP-103-302: A Phase 3, Randomized, Double-blind, Placebo-controlled, Parallel-group trial of Linaclotide Administered Orally for 26 weeks in Patients with Irritable Bowel Syndrome with Constipation

LIN-MD-31: A Phase 3, Randomized, Double-blind, Placebo-controlled, Parallel-group trial of Linaclotide Administered Orally for 12 weeks Followed by a 4-Week Randomized Withdrawal Period in Patients with Irritable Bowel Syndrome with Constipation

LIN-MD-01: A Phase 3, Randomized, Double-blind, Placebo-controlled, Parallel-group trial of Linaclotide Administered Orally for 12 weeks in Patients with Chronic Constipation

MCP-103-303: A Phase 3, Randomized, Double-blind, Placebo-controlled, Parallel-group trial of Linaclotide Administered Orally for 12 weeks Followed by a 4-Week Randomized Withdrawal Period in Patients with Constipation

This review will focus on the two studies (MCP-103-302 and LIN-MD-31) for the irritable bowel syndrome indication.

The original submission was submitted in eCTD and dated August 9, 2011.

The electronic submission is located at \\Cdsesub1\evsprod\NDA202811\0000.

The sponsor submitted a response to request dated October 28, 2011, to this reviewer's Information Request dated October 12, 2011.

The sponsor submitted a response to request dated November 07, 2011, to this reviewer's Information Request dated October 12, 2011.

The sponsor submitted a partial response to requests, dated, December 7, 2011, December 16, 2011 to this reviewer's Information Request dated October 12, 2011.

The sponsor submitted a response to request dated February 3, 2012, to this reviewer's Information Request dated December 7, 9, and 16, 2012.

The sponsor submitted a response to request dated March 2, 2012, to this reviewer's Information Request dated January 30, 2012.

The sponsor submitted a response to request dated March 5, 2012, to this reviewer's Information Request dated January 27, 2012.

The sponsor submitted a response, dated June 6, 2012 to this reviewer's Information Request dated May 23, 2012.

3. STATISTICAL EVALUATION

3.1 Evaluation of Efficacy

3.1.1 Study MCP-103-302

3.1.1.1 Study Design

This is a phase 3, randomized, double-blind, placebo-controlled, parallel-group trial of Linaclotide administered orally for 26 weeks in patients with Irritable Bowel Syndrome with Constipation (IBS-C). The trial was conducted in the U.S. (111 sites).

The objective of this trial was to determine the efficacy and safety of linaclotide administered to patients with irritable bowel syndrome with constipation (IBS-C).

Eligibility criteria are:

1. Males and females ≥ 18 years of age were included if they
2. Met the Rome II Criteria for IBS (i.e., they reported abdominal discomfort or pain that had ≥ 2 of the following features for ≥ 12 weeks, which need not be consecutive, in the 12 months preceding the Screening Visit:
    (a) relieved with defecation,
    (b) onset associated with a change in frequency of stool, and
    (c) onset associated with a change in form [appearance] of stool).
3. Patients must also have reported < 3 spontaneous bowel movements (SBMs) per week and reported ≥ 1 of the following symptoms for 12 weeks in the preceding 12 months:
    (a) straining during ≥ 25% of bowel movements (BMs),
    (b) lumpy or hard stools during ≥ 25% of BMs, and
    (c) a sensation of incomplete evacuation during > 25% of BMs.

Patients meeting these criteria were eligible if during the last 2 weeks of the Pretreatment Period they reported:

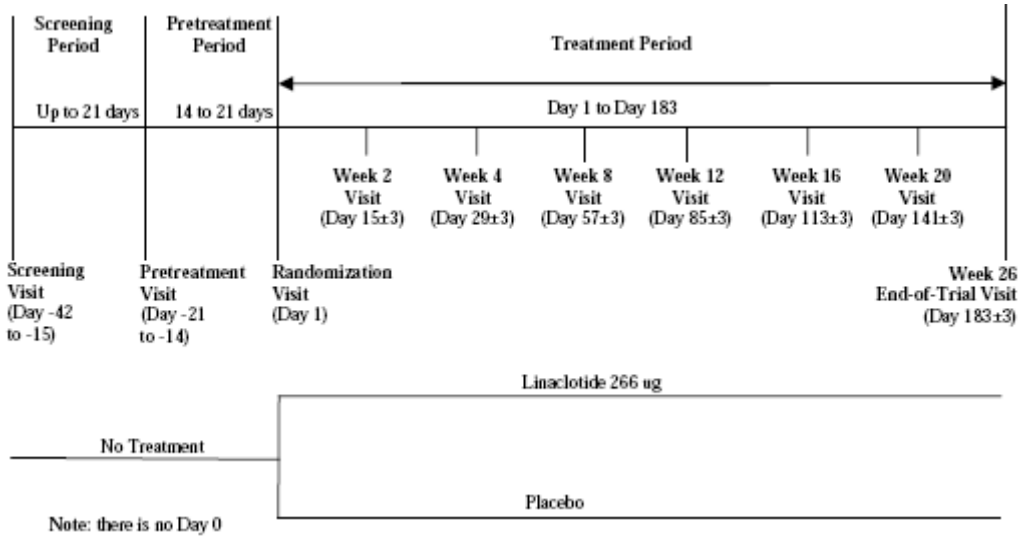
1. An average score for abdominal pain at its worst of ≥ 3.0, as reported in the IVRS using an 11-point numerical rating scale (NRS),
2. A mean score for abdominal pain at its worst of ≥ 3.0 for the Daily Patient Assessment of Abdominal Pain (11-point NRS),
3. < 3 complete SBMs (CSBMs) per week,
4. ≤ 5 SBMs per week, and
5. compliant with the IVRS.

Patients were excluded for any of the following reasons:

1. They reported loose (mushy) or watery stools in the absence of any laxative, enema, suppository, or prohibited medication for > 25% of BMs in the last 12 weeks preceding the Screening Visit;
2. They reported a Bristol Stool Form Scale (BSFS) score of 6 (loose, mushy stools) for > 1 SBM or a BSFS score of 7 (watery stool) with any SBM during the 14 days before the start of the Treatment Period;
3. They used Rescue Medicine (bisacodyl tablet or suppository) or any other laxative, suppository, or enema on the calendar day before or the calendar day of the start of the Treatment Period.

Patients were randomly assigned to 1 of 2 treatment groups (linaclotide dose of 266 LIN-MD-31/day or placebo) in a 1:1 ratio. The randomization assignments were generated in blocks of 4 so that each center would have a balanced distribution of patient assignments.

This trial consisted of 3 distinct periods



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The Screening Period (Day 042 through Day -15) started with the signature of the informed consent form (ICF) and lasted for up to 21 calendar days. During this period, patient eligibility for entry into the Pretreatment Period was determined. The end of the Screening Period coincided with the start of the Pretreatment Period.

The Pretreatment Period **(**Day -14 through Day -1**)** was defined as the 14 to 21 calendar days immediately before the Randomization Visit. During this period, patients provided the following information through daily calls to an interactive voice response system (IVRS):

• Daily Bowel Habits and Daily Patient Symptom Severity Assessments;
• Weekly Patient Symptom Severity and Weekly Patient Global Assessments;
• Use of Per-protocol Rescue Medicine or any other Laxatives, Suppositories, or Enemas.

Patients who satisfied all of the entry criteria were entered into the Treatment Period.

The Treatment Period **(**Day 1 through Day 183**)** began with randomization and lasted for 26 weeks. Patients were randomized to treatment with 266µg of linaclotide or with placebo (1:1), taken once daily in the morning at least 30 minutes before breakfast.

Patients continued to call the IVRS to provide their daily assessments (Daily Bowel Habits and Daily Patient Symptom Severity Assessments), weekly assessments (Weekly Patient Symptom Severity and Weekly Patient Global Assessments), and Use of Per-protocol Rescue Medicine or any other Laxatives, Suppositories, or Enemas. A number of quality of life (QOL) and patient-outcome assessments were performed at trial visits throughout the Treatment Period.

The primary efficacy parameters consisted of two components:
1) Abdominal Pain at its Worst and
2) CSBMs.

The daily patient assessments used to determine the primary efficacy parameter were as follows:

Patient assessment of Abdominal Pain at its Worst was collected daily by IVRS calls. The rating of Abdominal Pain at its Worst during the previous 24 hours on an 11-point NRS was provided by the patient answering the following question:

"How would you rate your abdominal pain at its worst over the last 24 hours? Enter a number from 0 to 10, where 0 represents no abdominal pain and 10 represents very severe abdominal pain."

Information needed to determine whether a BM was an SBM and a CSBM was collected daily during IVRS calls. Each day of the Pretreatment and Treatment Periods, the patient called into the IVRS system and provided the number of BMs he or she had since the

previous day's call. For each BM, the patient also provided the day the BM occurred and if the BM was associated with a sense of complete evacuation. (The patient was also asked to provide assessments of consistency and straining.) The patient was also asked if he or she took any Rescue Medicine since the previous day's call. For each type of Rescue Medicine taken (e.g., oral bisacodyl, bisacodyl suppository), or other laxatives, suppositories, or enemas, the patient was asked to provide the day it was taken.

An SBM was a BM that occurred in the absence of laxative, suppository, or enema use on the calendar day of the BM or the calendar day before the BM.

A CSBM was an SBM that was associated with a sense of complete evacuation.

The following IVRS patient assessments and questions were used to determine whether a BM was an SBM and whether an SBM was a CSBM.

• Day of any Rescue Medicine Use
"Have you taken any laxatives, suppositories, or enemas since yesterday's call at <IVRS-inserted time when this question was answered yesterday>?"
1=Yes
2=No

"Please enter 1=oral bisacodyl, 2=bisacodyl suppository, or 3=other laxatives, suppositories, or enemas."

"Was this rescue medication use today, or yesterday?"
1=Today
2=Yesterday

• The day of the BM
"How many bowel movements did you have since yesterday's call at <IVRS-inserted time when this question was answered yesterday>?"
"Was this bowel movement today, or yesterday?"
1=Today
2=Yesterday

• Whether the BM is associated with a sense of complete evacuation. This is assessed by
the patient answering the following IVRS question for each BM:
"Did you feel like you completely emptied your bowels?"
1=Yes
2=No

Patient assessment of Bloating was collected daily by IVRS calls. The rating of bloating during the previous 24 hours on an 11-point NRS was provided by the patient answering the following question:

"How would you rate your bloating over the last 24 hours? Enter a number from 0 to 10, where 0 represents no bloating and 10 represents very severe bloating."

Patient assessment of stool consistency was collected daily by IVRS calls. For each BM, stool consistency was assessed by the patient using the BSFS. The 7-point ordinal BSFS scale is provided below:

"Please refer to the laminated Bristol Stool Form Scale given to you. Please describe the consistency of the bowel movement using the following scale where:"
1=Separate hard lumps like nuts (difficult to pass)
2=Sausage shaped but lumpy
3=Like a sausage but with cracks on surface
4=Like a sausage or snake, smooth and soft
5=Soft blobs with clear-cut edges (passed easily)
6=Fluffy pieces with ragged edges, a mushy stool
7=Watery, no solid pieces (entirely liquid)

Patient assessment of straining was collected daily by IVRS calls. For each BM, degree of severity of straining was assessed by the patient using the following 5-point ordinal scale:

"How much did you strain during the bowel movement?"
1=Not at all
2=A little bit
3=A moderate amount
4=A great deal
5=An extreme amount

In addition to the primary and secondary efficacy assessments, the following efficacy assessments were used in determining the additional efficacy parameters.

Patient assessment of Abdominal Cramping was collected daily by IVRS calls. The rating of Abdominal Cramping during the previous 24 hours on an 11-point NRS was provided by the patient answering the following question:

"How would you rate your abdominal cramping over the last 24 hours? Enter a number from 0 to 10, where 0 represents no abdominal cramping and 10 represents very severe abdominal cramping."

Patient assessment of Abdominal Fullness was collected daily by IVRS calls. The rating of Abdominal Fullness during the previous 24 hours on an 11-point NRS was provided by the patient answering the following question:

"How would you rate your abdominal fullness over the last 24 hours? Enter a number from 0 to 10, where 0 represents no abdominal fullness and 10 represents very severe abdominal fullness."

3.1.1.2 Sponsor's Analysis

A total of 805 patients (402 patients in the 266µg linaclotide group and 403 patients in the placebo group) were randomized into the trial.

Number (%) of Patients Prematurely Discontinued During Treatment Period Randomized Population (Study MCP-103-302)

	Placebo (N=402) n (%)	Linaclotide (N=403) n (%)	Total (N=805) n (%)	p-value
Completed study	305 (75.7)	294 (73.1)	599 (74.4)	
Prematurely Discontinued	98 (24.3%)	108 (26.9)	206 (25.6)	0.4201
Reason for discontinuation				
Adverse event	10 (2.5)	41 (10.2)	51 (6.3)	<0.0001
Protocol violation	11 (2.7)	8 (2.0)	19 (2.4)	0.6436
Withdrawal of consent	26 (6.5)	24 (6.0)	50 (6.2)	0.8841
Lost to follow-up	13 (3.2)	18 (4.5)	31 (3.9)	0.3673
Insufficient therapeutic response	33 (8.2)	15 (3.7%)	48 (6.0)	0.0107
Other	5 (1.2)	2 (0.5)	7 (0.9)	0.4511

Complied from Table 14.1.3
p-values were obtained using Fisher's exact test.

As seen from the table above, of the 805 randomized patients, 599 (74%) completed the Treatment Period per protocol requirements. A total of 206 (26%) patients withdrew from the trial during the 26-week Treatment Period, with a similar percentage of withdrawals in the linaclotide and placebo groups...

A higher percentage of patients treated with linaclotide as compared with placebo discontinued due to an adverse event. A lower percentage of patients treated with linaclotide as compared with placebo discontinued due to insufficient therapeutic response.

3.1.1.2.1 Planned Analysis

The primary efficacy parameters assessed abdominal pain and BMs that met the criteria for CSBMs. There were 4 primary efficacy parameters:

1) 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responder: A weekly APC 3+1 Responder was a patient who had at least 3 CSBMs and an increase of at least 1 CSBM from baseline, and a decrease of at least 30% in the mean abdominal pain score, during a particular week. A 9/12 Week APC 3+1 Responder met these criteria for at least 9 of the first 12 weeks of the Treatment Period.

2) 9/12 Week CSBM 3+1 Responder: A weekly CSBM 3+1 Responder was a patient who had at least 3 CSBMs and an increase of at least 1 CSBM from baseline during a particular week. A 9/12 Week CSBM 3+1 Responder criteria met these criteria for at least 9 of the first 12 weeks of the Treatment Period

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3) 9/12 Week Abdominal Pain Responder: A weekly Abdominal Pain Responder was a patient who had a decrease of at least 30% from baseline in the mean abdominal pain score during a particular week. A 9/12 Week Abdominal Pain Responder met these criteria for at least 9 of the first 12 weeks of the Treatment Period.

4) 6/12 Week APC +1 Responder: A weekly APC +1 Responder was a patient who had an increase of at least 1 CSBM from baseline, and a decrease of at least 30% in the mean abdominal pain score, during a particular week. A 6/12 Week APC +1 Responder met these criteria for at least 6 of the first 12 weeks of the Treatment Period.

For each primary efficacy parameter, a patient had to have ≥ 4 complete IVRS calls for a particular Treatment Period week to be considered a responder for that week.

Secondary Efficacy Parameters:

There were 10 secondary efficacy parameters (8 change-from-baseline parameters and 2 responder parameters):

1. Change from Baseline in 12-week CSBM Frequency Rate,
2. Change from Baseline in.12-week SBM Frequency Rate,
3. Change from Baseline in 12-week Stool Consistency,
4. Change from Baseline in 12-week Severity of Straining,
5. Change from Baseline in 12-week Abdominal Pain,
6. Change from Baseline in 12-week Abdominal Discomfort,
7. Change from Baseline in 12-week Bloating,
8. Change from Baseline in.12-week Percent of Abdominal Pain-free Days,
9. 6/12 Week CSBM +1Responder, and
10. 6/12 Week Abdominal Pain Responder.

The overall type I family-wise error rate for testing the primary and secondary efficacy parameters was controlled at the 0.05 significance level using the following 5-step serial gatekeeping multiple comparisons procedure (MCP). Following this MCP, progression to the next step only occurred if all individual null hypotheses within a step were rejected and the previous step(s) were all rejected at the step-specific overall significance level. If all null hypotheses within a step were not rejected, the statistical tests corresponding to all subsequent steps were considered not statistically significant. All hypothesis tests were two-sided.

1. The first step tested the 4 primary efficacy parameters using a fixed sequential testing method. The 4 primary efficacy parameters were each tested at the 0.05 significance level in the following fixed sequence:

    1. 9/12 Week APC 3+1 Responder
    2. 9/12 Week CSBM 3+1 Responder
    3. 9/12 Week Abdominal Pain Responder
    4. 6/12 Week APC +1 Responder

    If a null hypothesis was not rejected (i.e., p-value > 0.05), all subsequent statistical tests were not considered statistically significant.

2. The second step tested the following 4 secondary parameters:
   - Change from baseline in 12-week CSBM Frequency Rate
   - Change from baseline in 12-week SBM Frequency Rate
   - Change from baseline in 12-week Stool Consistency
   - Change from baseline in 12-week Severity of Straining

   These 4 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure (22) to control for multiple parameters within this step.

3. The third step tested the following 3 secondary parameters:
   - Change from baseline in 12-week Abdominal Pain
   - Change from baseline in 12-week Abdominal Discomfort
   - Change from baseline in 12-week Bloating

   These 3 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure (22) to control for multiple parameters within this step.

4. The fourth step tested the following 2 secondary parameters:
   - 6/12 Week CSBM +1 Responder
   - 6/12 Week Abdominal Pain Responder

   These 2 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters within this step.

5. The fifth step tested the following single secondary parameter:
   - Change from baseline in 12-week Percent of Abdominal Pain-free Days

   This secondary parameter was tested using a type I error rate of 0.05.

Additional Efficacy Parameters:
The role of the additional efficacy parameters was to provide additional support for the primary and secondary efficacy parameters.

Approximately 800 patients (400 patients in the 266-LIN-MD-31 linaclotide group and 400 patients in the placebo group) were planned for randomization into the trial.

For this trial, the sample size was planned to be approximately 800 patients with 400 patients randomized to each of the two treatment groups: 266-LIN-MD-31 linaclotide and placebo.

This sample size was based on consideration of the overall efficacy results of study MCP-103-202, a 12-week, Phase 2b, double-blind, randomized study in 420 IBS-C patients. However, there were differences between this Phase 2b study and MCP-103-302 that were thought to possibly have an impact on responder rates, specifically the increased

availability of Rescue Medicine, the modification to the wording and scale of the daily Abdominal Pain assessment, and the revision to the responder definition.

Given the unknown impact on responder rates by these differences in study design between the Phase 2b study and MCP-103-302, it was deemed appropriate to have a larger sample size than was indicated by considering only the Phase 2b power calculation results. Table below summarizes the overall responder rate estimates for the primary efficacy parameters used in the power and sample size calculations for this trial.

Primary Efficacy Parameters' Power Calculations: Linaclotide Dose Estimates
Study MCP-103-302

Primary Efficacy Parameters	Placebo Estimate	Linaclotide Estimate	Nominal Power	Multiplicity Adjusted Power
1) 9/12 Week APC 3+1 Responder	10.0%	24.0%	> 99%	> 99%
2) 9/12 Week CSBM 3+1 Responder	12.5%	28.0%	> 99%	> 99%
3) 9/12 Week Abdominal Pain Responder	25.0%	45.3%	> 99%	> 99%
4) 6/12 Week APC +1 Responder	27.5%	49.3%	> 99%	> 99%

Note: The primary efficacy parameter rate estimates for placebo and linaclotide are based on Phase 2b responder rates from the placebo and linaclotide 266 (300) ug dose groups, respectively, incorporating the 4 complete IVRS call criterion. For each parameter, the nominal power is the probability of the p-value for the treatment group comparison being < 0.05. The multiplicity adjusted power estimates are based on 100,000 computer simulations which incorporate the fixed sequential testing method described in Section 9.7.1.3.4.

Copied from CSR Table 8.

Using the placebo and 266 (300) LIN-MD-31 linaclotide responder rate estimates from Phase 2b as presented in the table above, the adjustment for multiplicity, and based on a two-sample Chi-square two-sided test at the 5% significance level, with 400 randomized patients per treatment group arm, the power to reject all 4 primary efficacy parameters was > 99% (which was equivalent to rejecting the 6/12 Week APC +1 Responder parameter due to the fixed sequential testing procedure).

3.1.1.2.2 Treatment Group Comparability

The summary of results of comparability of treatment groups at baseline for all randomized patients is given in Appendix Tables 1 and 2.

As seen from Appendix Table 1, demographics and baseline characteristics were comparable between treatment groups. But, for gender, more patients were male in the placebo group (51 patients, 12.7%) compared to the linaclotide group (33 patients, 8.2%) (p = 0.0379).

As seen from Appendix Table 2, overall, baseline efficacy parameters were similar between the linaclotide and placebo groups.

Overall, concomitant medication use appeared to be similar between the placebo and linaclotide groups. However, the percentage of patients using propionic acid derivatives as concomitant medications was higher in the placebo group than in the linaclotide group (21.8% versus 16.4%).

Treatment compliance was > 96% for both treatment groups during the Treatment Period overall (97.2% and 96.8% in the placebo and linaclotide groups, respectively).

Overall, the percentage of patients who were IVRS compliant (had ≥ 80% complete calls within each day on ≥ 10 of the 14 days) during the 2-week Pretreatment Period was 92% and 94% in the placebo and linaclotide groups, respectively. During the 26-week Treatment Period, 63% and 64% of placebo and linaclotide patients were IVRS compliant (had ≥ 80% complete calls).

3.1.1.2.3 Sponsor's Analysis of Primary Efficacy Parameter

The primary endpoint was the number of patients who were 9/12 week APC 3+1 responders, defined as patients who were APC 3+1 responders for at least 9 of the 12 weeks of the treatment period. For each week in the treatment period, a weekly APC 3+1 responder was a patient who had at least 3 CSBMs for the week and an increase of at least 1 CSBM from baseline for that week, and also had a decrease of at least 30% in the mean abdominal pain score for that week.

The result from analysis of 9/12 week abdominal pain and CSBM (APC) 3+1 responders in the ITT population is given below.

Primary Efficacy Analysis: 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders—ITT Population
Study MCP-103-302

Description	Placebo (N=403) n (%)	Linaclotide (N=401) n (%)
Responder	12 (3.0)	51 (12.7)
Non-Responder	391 (97.0)	350 (87.3)
Difference in Responder Rate (Linaclotide - Placebo)	9.7	
Odds Ratio for Response (Linaclotide : Placebo)	4.65	
95% CI for Odds Ratio	(2.44, 8.84)	
p-value	< 0.0001	

Data Source: Section 14, Table 14.4.1.1A

A 9/12 week APC 3+1 responder was a patient who met the weekly APC 3+1 responder criteria for at least 9 of the first 12 weeks of the Treatment Period.

n = Number of patients within a specific category

N = Number of patients in the ITT Population

CI = Confidence interval

Odds ratio, 95% CI, and p-value were obtained from the CMH tests controlling for geographic region, comparing linaclotide versus placebo.

The p-value met the criterion for statistical significance based on the multiple comparison procedure.

As seen from the table above, the number and percentage of patients who were 9/12 week APC 3+1 Responders were greater for the linaclotide group when compared to placebo.

3.1.1.2.4 Sponsor's Analysis of Other Three Primary Efficacy Variable

The next two primary efficacy parameters (9/12 week CSBM 3+1 responders and 9/12 week abdominal pain responders) are the separate components of the first primary efficacy parameter. 9/12 week CSBM 3+1 responders was defined as patients who were CSBM 3+1 responders for at least 9 of the 12 weeks of the treatment period, and 9/12 week abdominal pain responders was defined as patients who were abdominal pain responders for at least 9/12 week of the treatment period.

The results from analysis of 9/12 week abdominal pain and CSBM (APC) 3+1 and 9/12 abdominal pain responder endpoints in the ITT population are given below.

Primary Efficacy Analysis: 9/12 Week CSBM 3+1 Responders
ITT Population
Study MCP-103-302

Description	Placebo (N=403) n (%)	Linaclotide (N=401) n (%)
Responder	20 (5.0)	72 (18.0)
Non-Responder	383 (95.0)	329 (82.0)
Difference in Responder Rate (Linaclotide - Placebo)	13.0	
Odds Ratio for Response (Linaclotide : Placebo)	4.19	
95% CI for Odds Ratio	(2.50, 7.03)	
p-value	< 0.0001	

Data Source: Section 14, Table 14.4.1.2A

A 9/12 week CSBM 3+1 responder was a patient who met the weekly CSBM 3+1 responder criteria for at least 9 of the first 12 weeks of the Treatment Period.

n = Number of patients within a specific category

N = Number of patients in the ITT Population

CI = Confidence interval

Odds ratio, 95% CI, and p-value were obtained from the CMH tests controlling for geographic region, comparing linaclotide versus placebo.

The p-value met the criterion for statistical significance based on the multiple comparison procedure.

Primary Efficacy Analysis: 9/12 Week Abdominal Pain Responders
ITT Population
Study MCP-103-302

Description	Placebo (N=403) n (%)	Linaclotide (N=401) n (%)
Responder	79 (19.6)	156 (38.9)
Non-Responder	324 (80.4)	245 (61.1)
Difference in Responder Rate (Linaclotide - Placebo)	19.3	
Odds Ratio for Response (Linaclotide : Placebo)	2.62	
95% CI for Odds Ratio	(1.91, 3.60)	
p-value	< 0.0001	

Data Source: Section 14, Table 14.4.1.3A

A 9/12 week Abdominal Pain Responder was a patient who met the weekly Abdominal Pain Responder criteria for at least 9 of the first 12 weeks of the Treatment Period.

n = Number of patients within a specific category

N = Number of patients in the ITT Population

CI = Confidence interval

Odds ratio, 95% CI, and p-value were obtained from the CMH tests controlling for geographic region, comparing linaclotide versus placebo.

The p-value met the criterion for statistical significance based on the multiple comparison procedure.

As seen from tables above, the percentage of 9/12 Week CSBM 3+1 responders in the linaclotide treatment group was statistically significantly higher than that in the placebo group). The percentage of 9/12 week abdominal pain responders in the linaclotide treatment group was also statistically significantly higher than that in the placebo group.

The fourth primary efficacy endpoint, 6/12 week APC +1 responder was defined as this patient met the weekly APC +1 responder criteria for at least 6 out of the 12 week of the treatment period. A weekly APC +1 responder was a patient who had an increase of at least 1 CSBM from baseline, and a decrease of at least 30% in the mean abdominal pain score, during a particular week.

The results from analysis of 6/12 week abdominal pain and CSBM (APC) +1 endpoint in the ITT population is given below.

Primary Efficacy Analysis: 6/12 Week APC+1 Responders - ITT Population Study MCP-103-302

Description	Placebo (N=403) n (%)	Linaclotide (N=401) n (%)
Responder	56 (13.9)	135 (33.7)
Non-Responder	347 (86.1)	266 (66.3)
Difference in Responder Rate (Linaclotide - Placebo)	19.8	
Odds Ratio for Response (Linaclotide : Placebo)	3.16	
95% CI for Odds Ratio	(2.22, 4.49)	
p-value	< 0.0001	

Data Source: Section 14, Table 14.4.1.4A

A 6/12 week APC +1 Responder was a patient who met the weekly APC +1 Responder criteria for at least 6 of the first 12 weeks of the Treatment Period.

n = Number of patients within a specific category

N = Number of patients in the ITT Population

CI = Confidence interval

Odds ratio, 95% CI, and p-value were obtained from the CMH tests controlling for geographic region, comparing linaclotide versus placebo.

The p-value met the criterion for statistical significance based on the multiple comparison procedure.

As seen from the table above, the percentage of 6/12 week APC +1 Responders was 33.7%) in the linaclotide group compared with 13.9% in the placebo group, ($p < 0.0001$).

3.1.1.2.5 Sponsor's Analyses of Secondary Variables

The secondary efficacy parameters based on the IVRS calls were:

- Change from baseline in 12-week CSBM frequency rate
- Change from baseline in 12-week SBM frequency rate
- Change from baseline in 12-week stool consistency
- Change from baseline in 12-week severity of straining
- Change from baseline in 12-week abdominal pain
- Change from baseline in 12-week abdominal discomfort
- Change from baseline in 12-week bloating
- Change from baseline in 12-week percent of abdominal pain-free days
- 6/12 week CSBM +1 responder (i.e., a patient who had an increase of at least 1 CSBM from baseline per week for 6 of the 12 weeks of treatment)

- 6/12 week abdominal pain responder

3.1.1.2.5.1 Change from Baseline in 12-week CSBM Frequency Rate

Summary of results of analysis of the change from baseline in 12-week CSBM frequency rate (i.e., weekly CSBM frequency rate over the 12-week treatment) is given below.

12-Week CSBM Frequency Rate – ITT Population
Study MCP-103-302

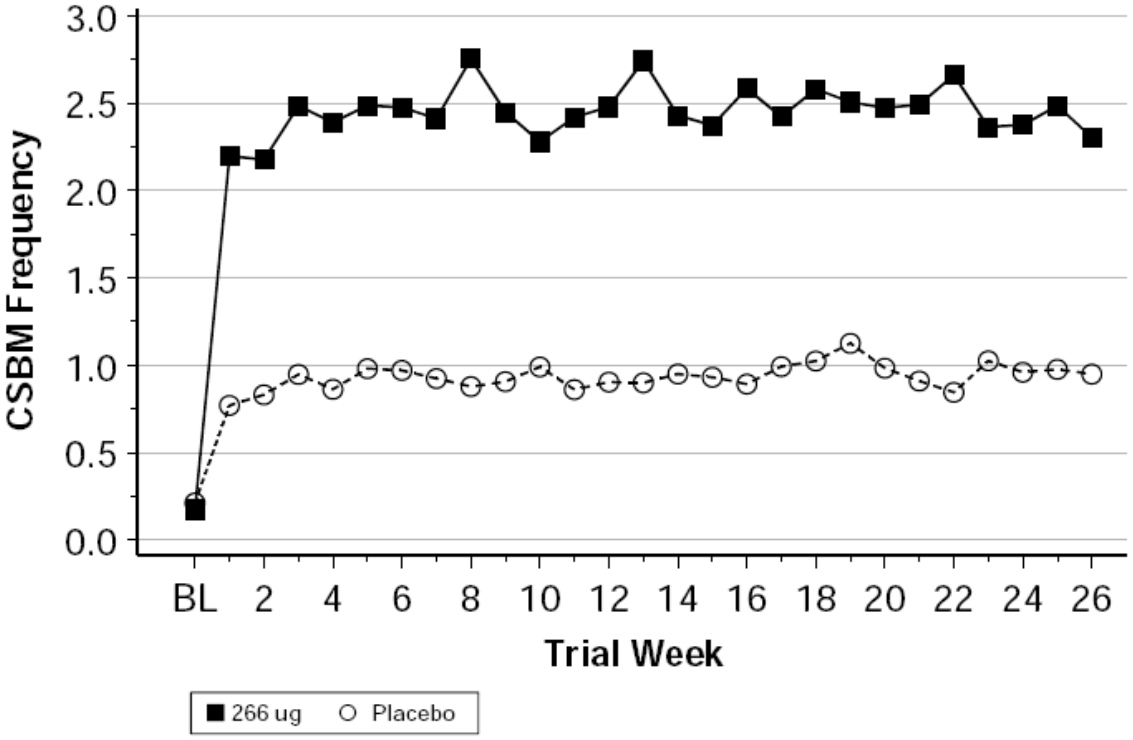
Visit	Statistic	Placebo (N=403)	Linaclotide (N=401)
Baseline	Mean	0.213	0.176
	SD	0.446	0.404
	SEM	0.022	0.020
	Median	0.000	0.000
	Min, Max	0.00, 2.88	0.00, 2.39
	n	403	401
Weeks 1-12	Mean	0.884	2.374
	SD	1.412	2.949
	SEM	0.070	0.147
	Median	0.252	1.421
	Min, Max	0.00, 9.72	0.00, 18.17
	n	403	401
Change from Baseline (ANCOVA Results)	LS Mean Change from Baseline (SE)	0.699 (0.122)	2.239 (0.122)
	LS Mean Difference (95% CI) [linaclotide - placebo]	1.540 (1.230, 1.850)	
	p-value[a]	< 0.0001	

Data source: Section 14, Table 14.4.2.1A
A patient's 12-week CSBM Frequency Rate is the CSBM rate (CSBMs/week) calculated over the first 12 weeks of the Treatment Period.
n = Number of patients in the ITT Population with analysis values at both baseline and during the Treatment Period
SEM = standard error of the mean, SE = standard error of LS Mean
[a] p-value is based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region as factors and baseline value as a covariate; p-value is less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean CSBM frequency rate during the treatment period is plotted by week and is given below.

Mean CSBM Rate during Each Week of Treatment Period (OC) – ITT
Study MCP-103-302



Data Source: Section 14, Table 14.4.2.1C
Weekly $p < 0.0001$ for linaclotide versus placebo during all weeks post-baseline; comparisons were based on an ANCOVA change from baseline model, with treatment group and geographic region as factors and baseline value as a covariate.

Copied from Figure 6.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 26-week treatment period.

3.1.1.2.5.2 Change from Baseline in 12-week SBM Frequency Rate

Summary of results of analysis of the change from baseline in 12-week SBM frequency rate (i.e., weekly SBM frequency rate over the 12-week treatment) is given below.

12-Week SBM Frequency Rate – ITT Population
Study MCP-103-302

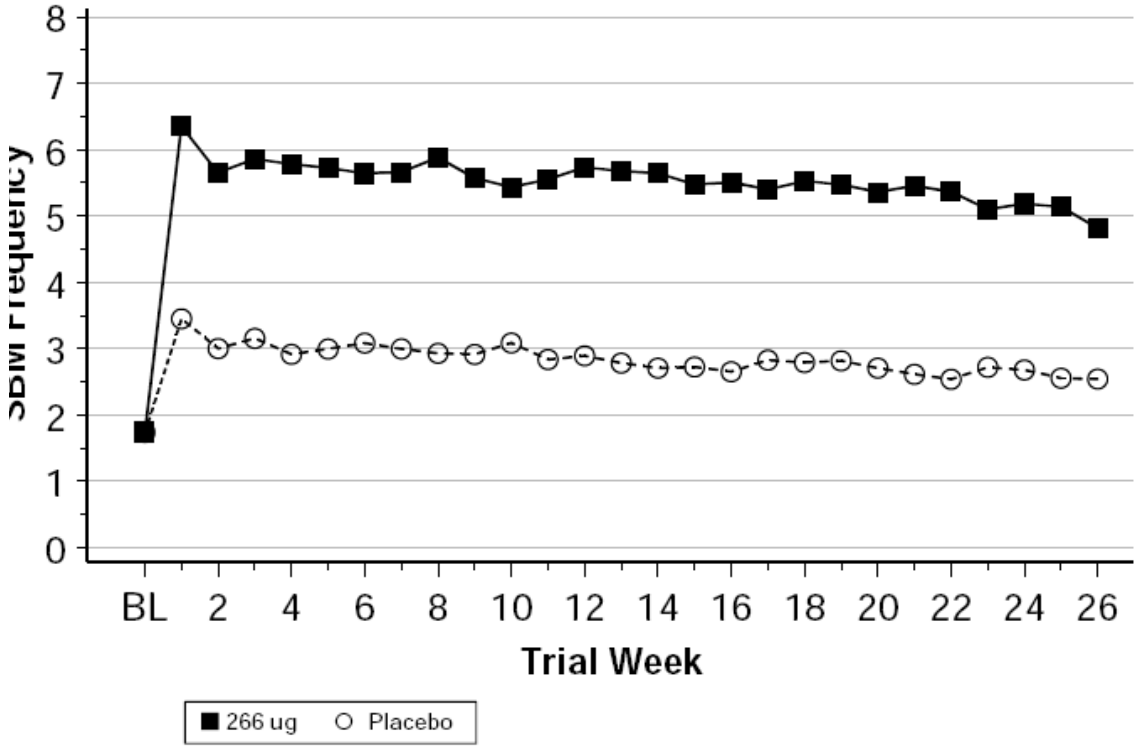
Visit	Statistic	Placebo (N=403)	Linaclotide (N=401)
Baseline	Mean	1.739	1.745
	SD	1.367	1.363
	SEM	0.068	0.068
	Median	1.461	1.457
	Min, Max	0.00, 5.36	0.00, 5.78
	n	403	401
Weeks 1-12	Mean	2.987	5.701
	SD	2.467	4.225
	SEM	0.123	0.211
	Median	2.511	4.870
	Min, Max	0.00, 23.65	0.00, 21.30
	n	403	401
Change from Baseline (ANCOVA Results)	LS Mean Change from Baseline (SE)	1.313 (0.176)	4.017 (0.176)
	LS Mean Difference (95% CI) [linaclotide - placebo]	2.704 (2.255, 3.153)	
	p-value[a]	< 0.0001	

Data source: Section 14, Table 14.4.2.2A
A patient's 12-week SBM Frequency Rate is the SBM rate (SBMs/week) calculated over the first 12 weeks of the Treatment Period.
n = Number of patients in the ITT Population with analysis values at both baseline and during the Treatment Period
SEM = standard error of the mean, SE = standard error of LS Mean
[a] p-value is based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region as factors and baseline value as a covariate; p-value is less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean SBM frequency rate during the treatment period is plotted by week and is given below.

Mean SBM Rate during Each Week of Treatment Period (OC) - ITT
Study MCP-103-302



Data Source: Section 14, Table 14.4.2.2C
Weekly $p < 0.0001$ for linaclotide versus placebo during all weeks post-baseline; comparisons were based on an ANCOVA change from baseline model, with treatment group and geographic region as factors and baseline value as a covariate.

Copied from Figure 7.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 26-week treatment period.

3.1.1.2.5.3 Change from Baseline in 12-week Stool Consistency

Summary of results of analysis of the change from baseline in 12-week stool consistency is given below.

12-Week Stool Consistency -ITT Population
Study MCP-103-302

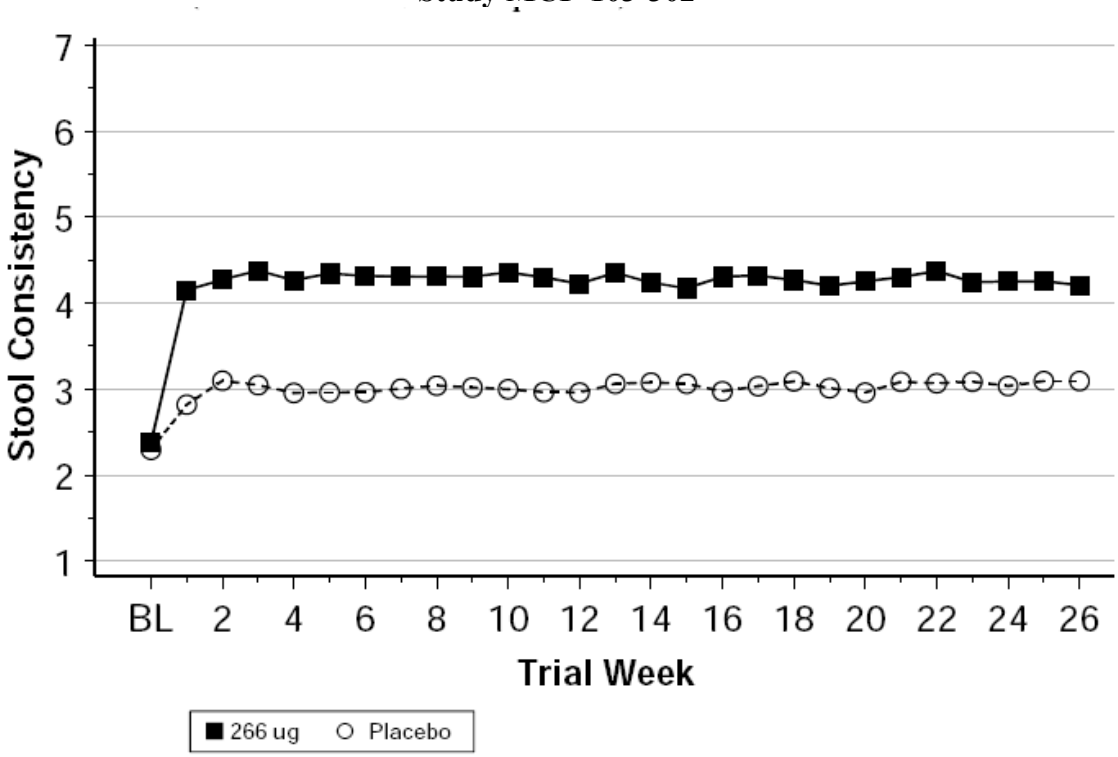
Visit	Statistic	Placebo (N=403)	Linaclotide (N=401)
Baseline	Mean	2.293	2.381
	SD	0.961	1.080
	SEM	0.053	0.059
	Median	2.000	2.000
	Min, Max	1.00, 6.00	1.00, 6.00
	n	332	338
Weeks 1-12	Mean	2.976	4.314
	SD	0.921	1.303
	SEM	0.051	0.071
	Median	3.000	4.367
	Min, Max	1.00, 6.71	1.00, 7.00
	n	332	338
Change from Baseline (ANCOVA Results)	LS Mean Change from Baseline (SE)	0.607 (0.064)	1.914 (0.063)
	LS Mean Difference (95% CI) [linaclotide - placebo]	1.307 (1.146, 1.468)	
	p-value[a]	< 0.0001	

Data source: Section 14, Table 14.4.2.3A
Stool consistency was measured daily using the seven-point ordinal BSFS. The patient's 12-week BSFS score is the average of the non-missing BSFS scores from the SBMs reported by the patient during the first 12-weeks of the Treatment Period.
n = Number of patients in the ITT Population with analysis values at both baseline and during the Treatment Period
SEM = standard error of the mean, SE = standard error of LS Mean
[a] p-value is based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region as factors and baseline value as a covariate; p-value is less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean stool consistency during the treatment period is plotted by week and is given below.

Mean Stool Consistency during Each Week of Treatment Period (OC) – ITT Population
Study MCP-103-302



Data Source: Section 14, Table 14.4.2.3C
Weekly $p < 0.0001$ for linaclotide versus placebo during all weeks post-baseline; comparisons were based on an ANCOVA change from baseline model, with treatment group and geographic region as factors and baseline value as a covariate.

Copied from Figure 8.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 26-week treatment period.

3.1.1.2.5.4 Change from Baseline in 12-week Severity of Straining

Summary of results of analysis of the change from baseline in 12-week severity of straining is given below.

12-Week Severity of Straining -ITT Population
Study MCP-103-302

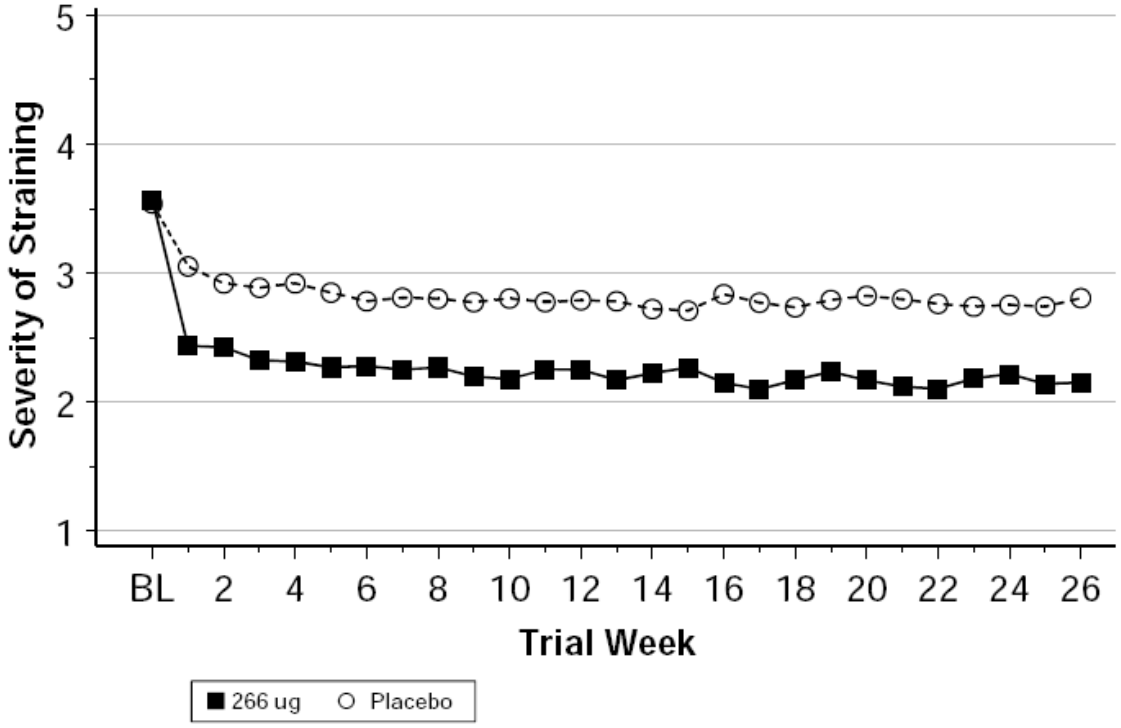
Visit	Statistic	Placebo (N=403)	Linaclotide (N=401)
Baseline	Mean	3.545	3.570
	SD	0.782	0.817
	SEM	0.043	0.044
	Median	3.600	3.600
	Min, Max	1.00, 5.00	1.00, 5.00
	n	332	338
Weeks 1-12	Mean	2.854	2.295
	SD	0.782	0.842
	SEM	0.043	0.046
	Median	2.834	2.222
	Min, Max	1.15, 4.86	1.00, 5.00
	n	332	338
Change from Baseline (ANCOVA Results)	LS Mean Change from Baseline (SE)	−0.663 (0.045)	−1.235 (0.044)
	LS Mean Difference (95% CI) [linaclotide - placebo]	−0.572 (−0.686, −0.459)	
	p-value[a]	< 0.0001	

Data source: Section 14, Table 14.4.2.4A
Severity of Straining was measured daily using a five-point ordinal scale (1 = not at all; 2 = a little bit; 3 = a moderate amount; 4 = a great deal; 5 = an extreme amount). The patient's straining score for the Treatment Period is the average of the non-missing straining scores from the SBMs reported by the patient during the first 12 weeks of the Treatment Period.
n = Number of patients in the ITT Population with analysis values at both baseline and during the Treatment Period
SEM = standard error of the mean, SE = standard error of LS Mean
[a] p-value is based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region as factors and baseline value as a covariate; p-value is less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean severity of straining stool consistency during the treatment period is plotted by week and is given below.

Change from Baseline in Mean Severity of Straining During Each Week of Treatment Period (OC) – ITT Population
Study MCP-103-302



Data Source: Section 14, Table 14.4.2.4C
Weekly $p < 0.0001$ for linaclotide versus placebo during all weeks post-baseline; comparisons were based on an ANCOVA change from baseline model, with treatment group and geographic region as factors and baseline value as a covariate.

Copied from Figure 9.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.1.2.5.5 Change from Baseline in 12-week Abdominal Pain

Summary of results of analysis of the change from baseline in 12-week abdominal pain is given below.

12-Week Abdominal Pain -ITT Population
Study MCP-103-302

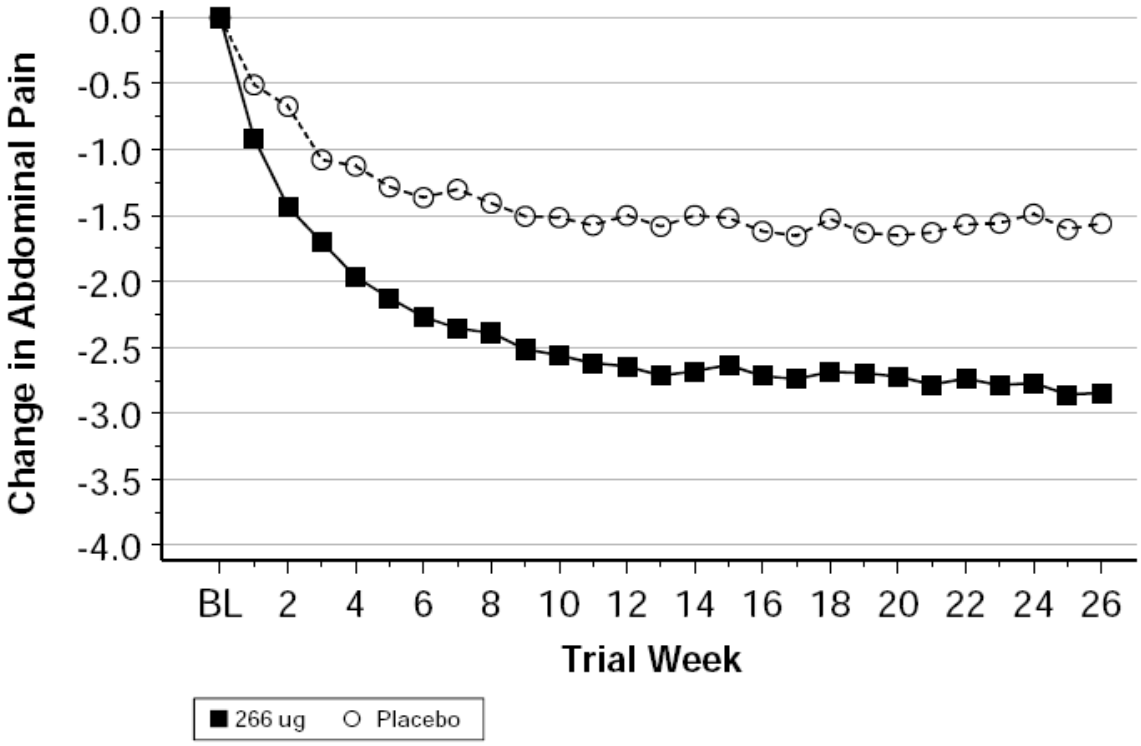
Visit	Statistic	Placebo (N=403)	Linaclotide (N=401)
Baseline	Mean	5.535	5.628
	SD	1.726	1.738
	SEM	0.086	0.087
	Median	5.333	5.417
	Min, Max	2.92, 10.00	2.92, 10.00
	n	403	401
Weeks 1-12	Mean	4.397	3.683
	SD	2.054	2.114
	SEM	0.102	0.106
	Median	4.073	3.400
	Min, Max	0.20, 9.74	0.00, 9.85
	n	403	401
Change from Baseline (ANCOVA Results)	LS Mean Change from Baseline (SE)	−1.070 (0.093)	−1.852 (0.093)
	LS Mean Difference (95% CI) [linaclotide - placebo]	−0.782 (−1.019, −0.545)	
	p-value[a]	< 0.0001	

Data source: Section 14, Table 14.4.2.5A
Abdominal pain was measured daily using an 11-point numerical rating scale. The patient's abdominal pain score for the Treatment Period is the average of the non-missing daily patient assessments of abdominal pain during the first 12 weeks of the Treatment Period.
n = Number of patients in the ITT Population with analysis values at both baseline and during the Treatment Period
SEM = standard error of the mean, SE = standard error of LS Mean
[a] p-value is based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region as factors and baseline value as a covariate; p-value is less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean abdominal pain during the treatment period is plotted by week and is given below.

Change from Baseline in Mean Abdominal Pain during Each Week of Treatment Period (OC) – ITT Population
Study MCP-103-302



Data Source: Section 14, Table 14.4.2.5C
Weekly $p < 0.0001$ for linaclotide versus placebo during all weeks post-baseline; comparisons were based on an ANCOVA change from baseline model, with treatment group and geographic region as factors and baseline value as a covariate.

Copied from Figure 10.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 26-week treatment period.

3.1.1.2.5.6 Change from Baseline in 12-week Abdominal Discomfort

Summary of results of analysis of the change from baseline in 12-week abdominal discomfort is given below.

12-Week Abdominal Discomfort -ITT Population
Study MCP-103-302

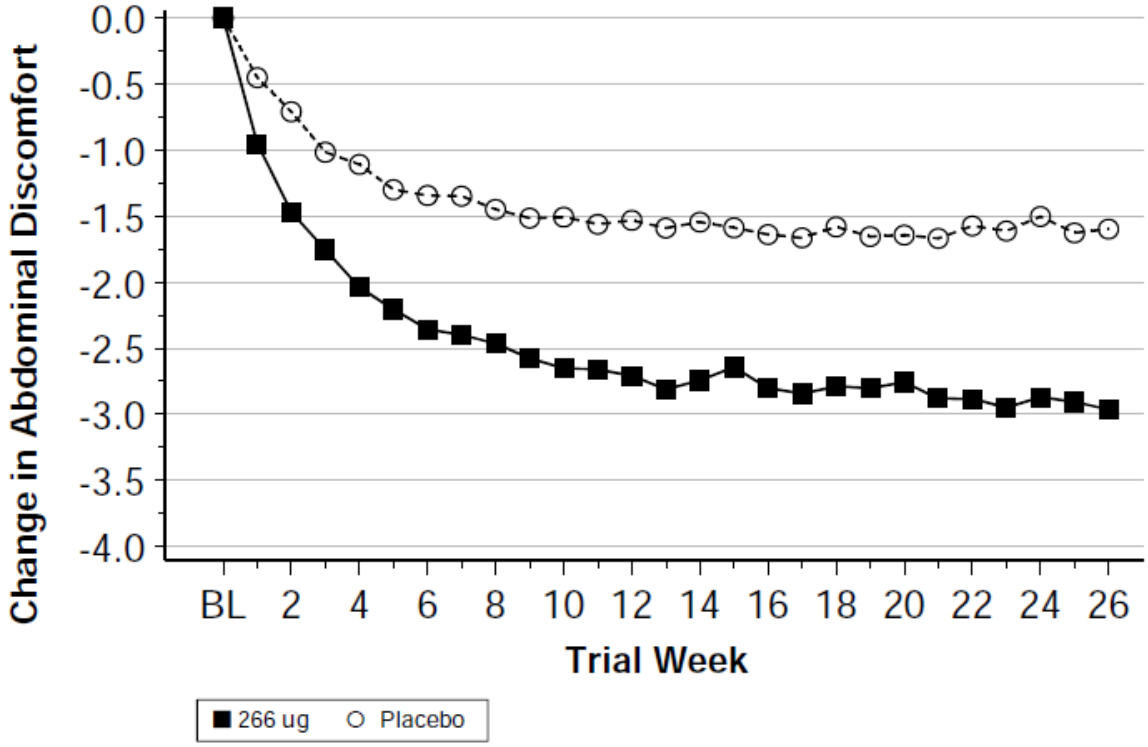
Visit	Statistic	Placebo (N=403)	Linaclotide (N=401)
Baseline	Mean	5.980	6.124
	SD	1.690	1.699
	SEM	0.084	0.085
	Median	5.786	6.067
	Min, Max	2.08, 10.00	2.47, 10.00
	n	403	401
Weeks 1-12	Mean	4.851	4.116
	SD	1.993	2.094
	SEM	0.099	0.105
	Median	4.759	3.914
	Min, Max	0.19, 10.00	0.00, 9.65
	n	403	401
Change from Baseline (ANCOVA Results)	LS Mean Change from Baseline (SE)	−1.103 (0.092)	−1.940 (0.092)
	LS Mean Difference (95% CI) [linaclotide - placebo]	−0.837 (−1.071, −0.603)	
	p-value[a]	< 0.0001	

Data source: Section 14, Table 14.4.2.6A
Abdominal discomfort was measured daily using an 11-point numerical rating scale. The patient's abdominal discomfort score for the Treatment Period is the average of the non-missing daily patient assessments of abdominal discomfort during the first 12 weeks of the Treatment Period.
n = Number of patients in the ITT Population with analysis values at both baseline and during the Treatment Period
SEM = standard error of the mean, SE = standard error of LS Mean
[a] p-value is based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region as factors and baseline value as a covariate; p-value is less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean abdominal discomfort during the treatment period is plotted by week and is given below.

Change from Baseline in Mean Abdominal Discomfort During Each Week of the Treatment Period (OC)--ITT Population)
Study MCP-103-302



Data Source: Section 14, Table 14.4.2.6C
Weekly $p < 0.0001$ for linaclotide versus placebo during all weeks post-baseline; comparisons were based on an ANCOVA change from baseline model, with treatment group and geographic region as factors and baseline value as a covariate.

Copied from Figure 11.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 26-week treatment period.

3.1.1.2.5.7 Change from Baseline in 12-week Bloating

Summary of results of analysis of the change from baseline in 12-week bloating is given below.

12-Week Bloating -ITT Population
Study MCP-103-302

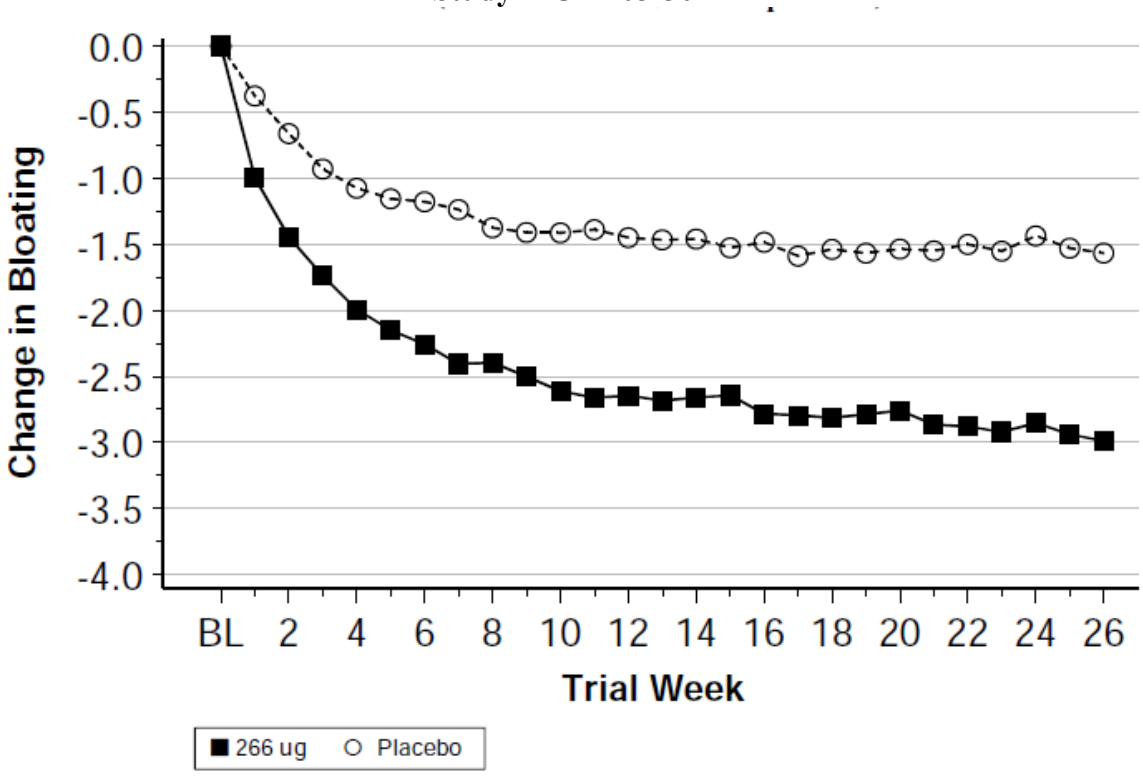
Visit	Statistic	Placebo (N=403)	Linaclotide (N=401)
Baseline	Mean	6.494	6.650
	SD	1.819	1.874
	SEM	0.091	0.094
	Median	6.500	6.636
	Min, Max	1.57, 10.00	0.00, 10.00
	n	403	401
Weeks 1-12	Mean	5.445	4.681
	SD	2.141	2.239
	SEM	0.107	0.112
	Median	5.340	4.556
	Min, Max	0.22, 10.00	0.00, 10.00
	n	403	401
Change from Baseline (ANCOVA Results)	LS Mean Change from Baseline (SE)	−1.032 (0.095)	−1.914 (0.094)
	LS Mean Difference (95% CI) [linaclotide - placebo]	−0.882 (−1.123, −0.641)	
	p-value[a]	< 0.0001	

Data source: Section 14, Table 14.4.2.7A
Bloating was measured daily using an 11-point numerical rating scale. The patient's bloating score for the Treatment Period is the average of the non-missing daily patient assessments of bloating during the first 12 weeks of the Treatment Period.
n = Number of patients in the ITT Population with analysis values at both baseline and during the Treatment Period
SEM = standard error of the mean, SE = standard error of LS Mean
[a] p-value is based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region as factors and baseline value as a covariate; p-value is less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean bloating during the treatment period is plotted by week and is given below.

Change from Baseline in Mean Bloating During Each Week of the Treatment Period (OC)--ITT Population)
Study MCP-103-302



Data Source: Section 14, Table 14.4.2.7C
Weekly $p < 0.0001$ for linaclotide versus placebo during all weeks post-baseline; comparisons were based on an ANCOVA change from baseline model, with treatment group and geographic region as factors and baseline value as a covariate.

Copied from Figure 12.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 26-week treatment period.

3.1.1.2.5.8 Change from Baseline in 12-week Percent of Abdominal Pain-free Days

Summary of results of analysis of the change from baseline in 12-week bloating is given below.

12-Week Percent of Abdominal Pain-free Days -ITT Population
Study MCP-103-302

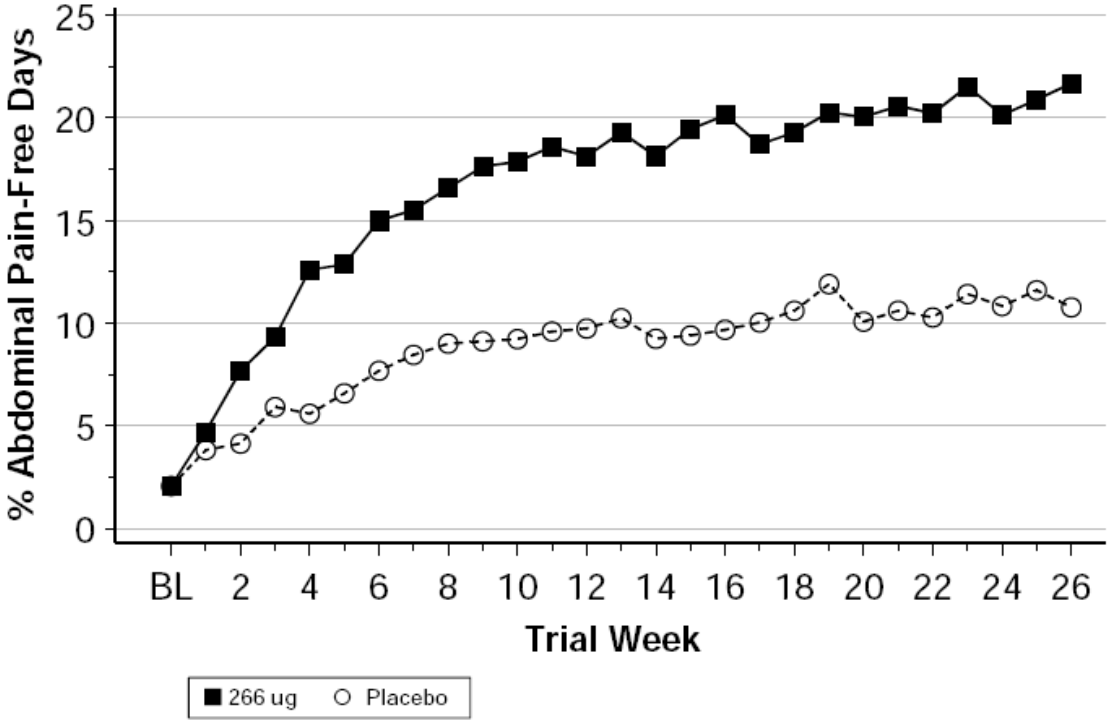
Visit	Statistic	Placebo (N=403)	Linaclotide (N=401)
Baseline	Mean	2.069	2.083
	SD	6.332	6.995
	SEM	0.315	0.349
	Median	0.00	0.00
	Q1, Q3	0.00, 0.00	0.00, 0.00
	Min, Max	0.00, 57.14	0.00, 53.85
	n	403	401
Weeks 1-12	Mean	6.893	12.570
	SD	17.349	24.213
	SEM	0.864	1.209
	Median	0.00	0.00
	Q1, Q3	0.00, 1.43	0.00, 13.04
	Min, Max	0.00, 95.18	0.00, 100.0
	n	403	401
Change from Baseline	Mean	4.825	10.487
	SD	16.638	23.422
	SEM	0.829	1.170
	Median	0.00	0.00
	Q1, Q3	0.00, 1.20	0.00, 7.27
	Min, Max	−55.89, 95.18	−51.35, 96.88
	n	403	401
	p-value[a]	0.0003	

Data source: Section 14, Table 14.4.2.10A
The percent of abdominal pain-free days was calculated as the number of abdominal pain-free days divided by the total number of days with non-missing daily abdominal pain at its worst assessments, multiplied by 100. The baseline percent and change from baseline in percent abdominal pain-free days were ranked first, respectively, and then transformed to normal scores for the ANCOVA analysis.
n = Number of patients in the ITT Population with analysis values at both baseline and during the Treatment Period
Q1 = 25th Percentile, Q3 = 75th Percentile, SEM = standard error of the mean, SE = standard error of LS Mean
a. p-value is based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region as factors and baseline value as a covariate, using rank-transformed normal scores; p-value is less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean percentage of abdominal pain-free days during the treatment period is plotted by week and is given below.

Mean Percent of Abdominal Pain-free During Each Week of Treatment Period (OC) – ITT Population
Study MCP-103-302



Data Source: Section 14, Table 14.4.2.10C
Weekly p-values for Weeks 1 and 3 were > 0.05 and weekly p-values for all other weeks were ≤ 0.0075 for linaclotide versus placebo during all weeks post-baseline; comparisons were based on an ANCOVA of rank-transformed normal scores of the change from baseline in abdominal pain-free days. The ANCOVA model had factors for treatment group and geographic region and rank-transformed normal scores of the baseline values as covariate.

Copied Figure 13.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 3 and was sustained over the following weeks of the 12-week treatment period.

3.1.1.2.5.9 6/12 week CSBM +1 Responder

This secondary efficacy endpoint was the number of patients who were 6/12-week CSBM +1 responders, defined as patients who were CSBM +1 responders for at least 6 of the 12 weeks of the treatment period. This is a component of the fourth primary efficacy parameter (6/12 week APC +1 responder). For each week in the treatment period, a weekly CSBM +1 responder was a patient who had at an increase of at least 1 CSBM from baseline for that week.

Summary of the results of analysis of 6/12 week CSBM +1 responder is given below.

Secondary Efficacy Analysis: 6/12 Week CSBM + 1 Responders -ITT
Study MCP-103-302

Description	Placebo (N=403) n (%)	Linaclotide (N=401) n (%)
Responder	91 (22.6)	191 (47.6)
Non-Responder	312 (77.4)	210 (52.4)
Difference in Responder Rate (Linaclotide - Placebo)	25.1	
Odds Ratio for Response (Linaclotide : Placebo)	3.11	
95% CI for Odds Ratio	(2.29, 4.21)	
p-value	< 0.0001	

Data Source: Section 14, Table 14.4.2.8A
A 6/12 week CSBM +1 responder was a patient who met the weekly CSBM +1 responder criteria for at least 6 of the first 12 weeks of the Treatment Period.
n = Number of patients within a specific category
N = Number of patients in the ITT Population
CI = Confidence interval
Odds ratio, 95% CI, and p-value were obtained from the CMH tests controlling for geographic region, comparing linaclotide versus placebo.
The p-value met the criterion for statistical significance based on the MCP.

As seen from the table above, the percentage of responders in the linaclotide treatment group was significantly greater than that in the placebo group.

3.1.1.2.5.10 6/12 Week Abdominal Pain Responder

This secondary efficacy endpoint was the number of patients who were 6/12-week abdominal pain responders, defined as patients who were abdominal pain responders for at least 6 of the 12 weeks of the treatment period. This is a component of the fourth primary efficacy parameter (6/12 week APC +1 responder). For each week in the treatment period, a weekly abdominal pain responder was a patient who had at a decrease of at least 30% in abdominal pain score from baseline for that week.

Summary of the results of analysis of 6/12 week abdominal pain responder is given below.

Secondary Efficacy Analysis: 6/12 Week Abdominal Pain Responders - ITT
Study MCP-103-302

Description	Placebo (N=403) n (%)	Linaclotide (N=401) n (%)
Responder	139 (34.5)	196 (48.9)
Non-Responder	264 (65.5)	205 (51.1)
Difference in Responder Rate (Linaclotide - Placebo)	14.4	
Odds Ratio for Response (Linaclotide : Placebo)	1.82	
95% CI for Odds Ratio	(1.37, 2.42)	
p-value	< 0.0001	

Data Source: Section 14, Table 14.4.2.9A

A 6/12 week Abdominal Pain Responder was a patient who met the weekly Abdominal Pain Responder criteria for at least 6 of the first 12 weeks of the Treatment Period.

n = Number of patients within a specific category

N = Number of patients in the ITT Population

CI = Confidence interval

Odds ratio, 95% CI, and p-value were obtained from the CMH tests controlling for geographic region, comparing linaclotide versus placebo.

The p-value met the criterion for statistical significance based on the MCP.

As seen from the table above, percentage of responders in the linaclotide treatment group was significantly greater than that in the placebo group.

3.1.1.3 Reviewer's Comments and Evaluation

3.1.1.3.1 Sensitivity Analyses of 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders

Per request, the sponsor performed sensitivity analyses of 9/12 week abdominal pain and CSBM (APC) 3 + 1 responder.

The results from sensitivity analyses of f 9/12 week abdominal pain and CSBM (APC) 3 + 1 responder are given below.

9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders
Study MCP-103-302

Analysis	PLA	LIN	Diff (RFX-PLA)	P-value
(LOCF)	18/403 (4.5%)	68/401 (17.0%)	12.5%	<0.0001
Completed Case	11/245 (4.5%)	47/253 (18.6%)	14.1%	<0.0001
Observed Case	12/400 (3.0%)	51/394 (12.9%)	9.9%	<0.0001
Worst Case 1	11/403 (2.7%)	47/401 (11.7%)	9.0%	<0.0001
Worst Case 2	169/403 (41.9%)	47/401 (11.7%)	-30.2%	<0.00001
Worst Case 3	58/403 (14.4%)	51/401 (11.7%)	-1.7%	0.5056
Multiple Imputation	3.5%	16.4%	12.9%	<0.0001

Complied from Tables 14.4.1.1D-14.4.1.1I and 14.4.1.1K
P- values were obtained from the CMH tests controlling for geographic region.
The complete case analysis includes only those patients who complete at least 4 IVRS calls for each of the first 12 weeks of treatment.
The observed case analysis includes only those patients who complete at least 4 IVRS calls for at least one of the first 12 weeks of treatment.
For worst case analysis 1, patients must complete at least 4 IVARS calls for each of the first 12 weeks of treatment.
For worst case analysis 2, patients who do not complete at least 4 IVRS call for each of the first 12 weeks of treatment are handled as follows: patients randomized to Linaclotide are non-responders, while patients who are randomized to placebo are considered responders.
For worst case analysis 3, for those weeks where patients do not complete at least 4 IVRS calls, patients randomized to Linaclotide are non-responders, while patients who are randomized to placebo are considered responders.

The sponsor applied the following definition for Worst Case 1:

- Worst Case 1: If a patient has less than 4 complete calls for any of the first 12 Treatment Period weeks, that patient will be assumed to be “failed” and defined as a nonresponder for the trial.

Under the Worst Case 1 method, if a patient had less than 4 complete IVRS calls in any one of Treatment Period weeks 1 - 12, that patient was defined as a primary efficacy endpoint non-responder.

In contrast, for the primary efficacy endpoint analysis in the IBS-C trials if a patient had less than 4 complete IVRS calls for one or more of the Treatment Period weeks 1 - 12, that patient would be defined as a weekly non-responder for those particular weeks, but could still be a primary efficacy endpoint responder.

As such, the number of patients classified as primary efficacy endpoint non-responders under the Worst Case 1 method will be higher than the primary efficacy analysis method as only those patients who have at least 4 complete IVRS calls in all 12 Treatment Period

weeks could potentially be primary efficacy endpoint responders under the Worst Case 1 method.

The sponsor's worst case 1 analysis is one of "worst cast" analyses. It is more conservative than the sponsor's analysis.

As seen from the table above, for 9/12 week abdominal pain and CSBM (APC) 3 + 1 responder was shown by a significantly greater proportion of subjects taking linaclotide compared with subjects taking placebo in the worst cast 1 analysis. The result was similar to that obtained by the sponsor.

The sensitivity analysis using observed cases analysis showed similar results.

3.1.1.3.2 Subgroup Analyses of 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders for gender, age, race, BMI at baseline, and abdominal pain at baseline.

The summary of results of subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders is given below.

Subgroup Analyses of Proportion of 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders (Study MCP-103-302)

Subgroup	Placebo	Linaclotidde	Diff (LIN-PLA)	95% CI
Gender				
Male	3/51 (5.9%)	5/33 (15.2%)	9.3%	(8.8%, 9.7%)
Female	9/352 (2.6%)	46/368 (12.5%)	9.9%	(9.8%, 10.1%)
Age				
<65	12/386 (3.1%)	47/378 (12.4%)	9.3%	(9.2%, 9.4%)
≥65	0/17 (0.0%)	4/23 (17.4%)	17.4%	(16.9%, 17.9%)
Race				
White	9/311 (2.9%)	43/316 (13.6%)	10.7%	(10.6%, 10.8%)
Black	2/78 (2.6%)	7/70 (10.0%)	7.4%%	(7.2%, 7.7%)
Other	1/14 (7.1%)	1/15 (6.7%)	-0.4%	(-1.1%, 0.1%)
BMI at baseline				
< 30 kg/m^2	7/285 (2.5%)	35/280 (12.5%)	10.0%	(9.9%, 10.2%)
≥ 30 kg/m^2	5/118 (4.2%)	16/121 (13.2%)	9.0%	(8.8%, 9.2%)
Abdominal Pain at Baseline				
< 5	4/176 (2.3%)	21/165 (12.7%)	10.4%	(10.3%, 10.6%)
≥ 5 < 8	7/185 (3.8%)	27/189 (14.3%)	10.5%	(10.3%, 10.7%)
≥ 8	1/42 (2.4%)	3/47 (6.4%)	4.0%	(-3.7%, 4.3%)

Compiled by this reviewer from Table 14.4.1.1J

As seen from the table above, 9 /12 week abdominal pain and CSBM(APC) 3 + 1 responder were reported by higher proportion of linaclotide subjects for gender, age, race, and BMI at baseline, abdominal pain at baseline (<5 and ≥ 5 < 8).

3.1.1.3.3 Adequate Relief of Abdominal Pain and CSBM (APC) 3 + 1 Responder Rates by Week through Week 26

As per request, the sponsor provided tabulation of number of subjects with adequate relief of abdominal pain and CSBM(APC) 3 + 1 by week through week 26 for ITT population (see below).

Weekly Abdominal Pain and CSBM (APC) 3 + 1 Responder Rate by Treatment Group Intention-to-Treat Population Study MCP-103-302

	StudyMCP-103-302			
	PLA	**LIN**	**Diff** (LIN-PLA)	Chi-square p-value
Week 1	16/403 (4.0%)	51/401 (12.7%)	8.7%	<0.0001
Week 2	20/403 (5.0%)	81/401 (20.2%)	15.2%	<0.0001
Week 3	33/403 (8.2%)	88/401 (21.9%)	13.7%	<0.0001
Week 4	31/403 (7.7%)	95/401 (23.7%)	16.0%	<0.0001
Week 5	37/403 (9.2%)	92/401 (22.9%)	13.7%	<0.0001
Week 6	33/403 (8.2%)	97/401 (24.2%)	16.0%	<0.0001
Week 7	36/403 (8.9%)	95/401 (23.7%)	14.8%	<0.0001
Week 8	27/403 (6.7%)	103/401 (25.7%)	19.0%	<0.0001
Week 9	33/403 (8.2%)	85/401 (21.2%)	13.0%	<0.0001
Week 10	40/403 (9.9%)	89.401 (22.2%)	12.3%	<0.0001
Week 11	33/403 (8.2%)	86/401 (21.4%)	13.2%	<0.0001
Week 12	40/403 (9.9%)	103/401 (25.7%)	15.8%	<0.0001
Week 13	38/403 (9.4%)	96/401 (23.9%)	14.5%	<0.0001
Week 14	35/403 (8.7%)	97/401 (24.2%)	15.5%	<0.0001
Week 15	36/403 (8.9%)	86/401 (21.4%)	12.5%	<0.0001
Week 16	35/403 (8.7%)	92/401 (22.9%)	14.2%	<0.0001
Week 17	41/403 (10.2%)	92/401 (22.9%)	12.7%	<0.0001
Week 18	38/403 (9.4%)	87/401 (21.7%)	12.3%	<0.0001
Week 19	43/403 (10.7%)	92/401 (22.9%))	12.2%	<0.0001
Week 20	36/403 (8.9%)	83/401 (20.7%)	11.8%	<0.0001
Week 21	33/403 (8.2%)	88/401 (21.9%)	13.7%	<0.0001
Week 22	32/403 (7.9%)	95/401 (23.7%)	15.8%	<0.0001
Week 23	36/403 (8.9%)	86/401 (21.4%)	12.5%	<0.0001
Week 24	40/403 (9.9%)	82/401 (20.4%)	10.5%	<0.0001
Week 25	37/403 (9.2%)	91/401 (22.7%)	13.5%	<0.0001
Week 26	27/403 (6.7%)	79/401 (19.7%)	13.0%	<0.0001

Compiled by this reviewer from Table 14.4.1.1C.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, greater proportions of subjects at almost every week during the course of the 26- week study in the linaclotide group compared with subjects in the placebo group was observed.

3.1.1.3.4 Adequate Relief of Abdominal Pain and CSBM (APC) + 1 Responder Rates by Week through Week 26

As per request, the sponsor provided tabulation of number of subjects with adequate relief of abdominal pain and CSBM (APC) + 1 by week through week 26 for ITT population (see below).

Weekly Abdominal Pain and CSBM (APC) + 1 Responder Rate
by Treatment Group
Intention-to-Treat Population
Study MCP -103-302

	PLA	**LIN**	**Diff** (LIN-PLA)	Chi-square p-value
Week 1	29/403 (7.2%)	78/401 (19.5%)	12.3%	<0.0001
Week 2	46/403 (11.4%)	115/401 (28.7%)	17.3%	<0.0001
Week 3	63/403 (15.6%)	130/401 (32.4%)	16.8%	<0.0001
Week 4	56/403 (13.9%)	131/401 (32.7%)	18.8%	<0.0001
Week 5	73/403 (18.1%)	141/401 (35.2%)	17.1%	<0.0001
Week 6	70/403 (17.4%)	148/401 (36.9%)	19.5%	<0.0001
Week 7	67/403 (16.6%)	134/401 (33.4%)	16.8%	<0.0001
Week 8	64/403 (15.9%)	133/401 (33.2%)	17.3%	<0.0001
Week 9	59/403 (14.6%)	137/401 (34.2%)	19.6%	<0.0001
Week 10	70/401 (17.4%)	132/401 (32.9%)	15.5%	<0.0001
Week 11	68/403 (16.9%)	133/401 (33.2%)	16.3%	<0.0001
Week 12	61/403 (15.1%)	137/401 (34.2%)	19.1%	<0.0001
Week 13	60/404 (14.9%)	132/401 (32.9%)	18.0%	<0.0001
Week 14	56/403 (13.9%)	125/401 (31.2%)	17.3%	<0.0001
Week 15	62/403 (15.4%)	123/401 (30.7%)	15.3%	<0.0001
Week 16	66/403 (16.4%)	135/401 (33.7%)	17.3%	<0.0001
Week 17	57/403 (14.1%)	129/401 (32.2%)	18.1%	<0.0001
Week 18	67/403 (16.6%)	121/401 (30.2%)	13.6%	<0.0001
Week 19	64/403 (15.9%)	122/401 (30.4%)	14.5%	<0.0001
Week 20	68/403 (16.9%)	123/401 (30.7%)	13.8%	<0.0001
Week 21	60/403 (14.9%))	130/401 (32.4%)	17.5%	<0.0001
Week 22	54/403 (13.4%)	125/401 (31.2%)	17.8%	<0.0001
Week 23	60/403 (14.9%)	119/401 (29.7%)	14.8%	<0.0001
Week 24	59/403 (14.6%)	114/401 (28.4%)	13.8%	<0.0001
Week 25	60/403 (14.9%)	123/401 (30.7%)	15.8%	<0.0001
Week 26	48/403 (11.9%)	104/401 (25.9%)	14.0%	<0.0001

Compiled by this reviewer from Table 14.4.1.4C.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, greater proportions of subjects at almost every week during the course of the 26- week study in the linaclotide group compared with subjects in the placebo group was observed.

3.1.1.3.5 Monthly Abdominal Pain and CSBM (APC) Responder Rate

The monthly responder is defined that a subject be a weekly responder for at least 2 of the 4 treatment period weeks for that month.

This reviewer performed analyses of abdominal pain and CSBM (APC) by month. Subject with missing monthly responder at a specific month was assumed to be "failure" for that month.

The results from reviewer's analyses of abdominal pain and CSBM (APC) by month are given below.

Monthly Abdominal Pain and CSBM (APC) 3 + 1 Responder Rate
by Treatment Group
MCP-103-302
Intention-to-Treat Population

	PLA	**LIN**	**Diff** (LIN-PLA)	Chi-square p-value
Month 1	28/403 (6.9%)	97/401 (24.2%)	17.2%	<0.0001
Month 2	35/403 (8.7%)	115/401 (28.7%)	20.0%	<0.0001
Month 3	43/403 (10.7%)	107/401 (26.7%)	16.0%	<0.0001
Month 4	41/403 (10.2%)	100/403 (24.9%)	14.7%	<0.0001
Month 5	47/403 (11.7%)	98/401 (24.4%)	12.7%	<0.0001
Month 6	40/403 (9.9%)	99/401 (24.7%)	14.8%	<0.0001

Compiled by this reviewer from Table 14.4.3.24C.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the tables above, for monthly abdominal pain and CSBM (APC) 3 + 1 responders, greater proportions of subjects at every month during the course of the 6-month study in the linaclotide group compared with subjects in the placebo group was observed.

Monthly Abdominal Pain and CSBM (APC) + 1 Responder Rate
by Treatment Group
MCP-103-302
Intention-to-Treat Population

	PLA	**LIN**	**Diff** (LIN-PLA)	Chi-square p-value
Month 1	36/403 (13.9%)	136/401 (33.9%)	20.0%	<0.0001
Month 2	72/403 (17.9%)	152/401 (37.9%)	20.0%	<0.0001
Month 3	75/403 (18.6%)	154/401 (38.4%)	19.8%	<0.0001
Month 4	69/403 (17.1%)	143/401 (35.4%)	18.6%	<0.0001
Month 5	73/403 (18.1%)	143/401 (35.4%)	17.6%	<0.0001
Month 6	65/403 (16.1%)	134/401 (33.4%)	17.3%	<0.0001

Compiled by this reviewer from Table 14.4.3.27C.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the tables above, for monthly abdominal pain and CSBM (APC) + 1 responder, greater proportions of subjects at every month during the course of the 24-week study in the linaclotide group compared with subjects in the placebo group was observed.

3.1.1.3.6 Sustained Efficacy – Monthly Abdominal Pain and CSBM (APC) Responder

3.1.1.3.6.1 Sustained Efficacy – At Last 2 of 3 Months

For sustained efficacy, the commonly used primary efficacy endpoint for IBS is "overall responder." A subject was considered an overall responder if the subject was a monthly responder for at least two out of any three months during 12-week study.

This reviewer performed analysis of overall responder for abdominal pain and CSBM (APC) 3 + 1 and abdominal pain and CSBM (APC) + 1. The results are given below.

Reviewer's Overall Responder Analysis by Treatment Group
MCP-103-302
Intention-to-Treat Population

Endpoint	PLA N=403	LIN n=401	Diff (LIN-PLA)	p-value
Abdominal Pain and CSBM (APC) 3 + 1 ≥ 2 Months	32 (7.9%)	106 (26.4%)	18.5%	<0.0001
Abdominal Pain and CSBM (APC) + 1 ≥ 2 Months	65 (16.1%)	150 (37.4%)	21.3%	<0.0001

Compiled by this reviewer from Table 14.4.3.24A and Table 14.-4.3.27A.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, for overall responder for abdominal pain and CSBM (APC) 3 + 1 and abdominal pain and CSBM (APC) + 1, greater proportions of subjects in the linaclotide group compared with subjects in the placebo group was observed.

3.1.1.3.6.2 Sustained Efficacy – All 3 Months

For sustained efficacy, a subject was considered an overall responder if the subject was a monthly responder for all 3 months during 12-week study. This definition is more stringent than previous definition for overall responder (at least 2 of 3 months).

This reviewer performed analysis of overall responder for abdominal pain and CSBM (APC) 3 + 1 and abdominal pain and CSBM (APC) + 1. The results are given below.

Reviewer's Overall Responder Analysis by Treatment Group
MCP-103-302
Intention-to-Treat Population

Endpoint	PLA N=403	LIN n=401	Diff (LIN-PLA)	p-value
Abdominal Pain and CSBM (APC) 3 + 1 = 3 Months	15 (3.7%)	66 (16.5%)	12.8%	<0.0001
Abdominal Pain and CSBM (APC) + 1 = 3 Months	30 (7.4%)	98 (24.4%)	17.0%	<0.0001

Compiled by this reviewer
P-values were obtained by the Fisher's Exact test.

As seen from the table above, for overall responder for abdominal pain and CSBM (APC) 3 + 1 and abdominal pain and CSBM (APC) + 1, greater proportions of subjects in the linaclotide group compared with subjects in the placebo group was observed.

3.1.1.3.7 Reviewer's Comments on Sponsor's Controlling for Multiplicity for Primary and Secondary Efficacy Parameter

The sponsor used 5-step serial gatekeeping multiple comparison procedure to control type 1 family-wise error rate for testing the primary and secondary efficacy parameters.

The overall type I family-wise error rate for testing the primary and secondary efficacy parameters was controlled at the 0.05 significance level using the following 5-step serial gatekeeping multiple comparisons procedure (MCP). Following this MCP, progression to the next step only occurred if all individual null hypotheses within a step were rejected and the previous step(s) were all rejected at the step-specific overall significance level. If all null hypotheses within a step were not rejected, the statistical tests corresponding to all subsequent steps were considered not statistically significant. All hypothesis tests were two-sided.

1. The first step tested the 4 primary efficacy parameters using a fixed sequential testing method. The 4 primary efficacy parameters were each tested at the 0.05 significance level in the following fixed sequence:

    1. 9/12 Week APC 3+1 Responder
    2. 9/12 Week CSBM 3+1 Responder
    3. 9/12 Week Abdominal Pain Responder
    4. 6/12 Week APC +1 Responder

    If a null hypothesis was not rejected (i.e., p-value > 0.05), all subsequent statistical tests were not considered statistically significant.

2. The second step tested the following 4 secondary parameters:
    - Change from baseline in 12-week CSBM Frequency Rate
    - Change from baseline in 12-week SBM Frequency Rate
    - Change from baseline in 12-week Stool Consistency

- Change from baseline in 12-week Severity of Straining

These 4 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure (22) to control for multiple parameters within this step.

3. The third step tested the following 3 secondary parameters:
   - Change from baseline in 12-week Abdominal Pain
   - Change from baseline in 12-week Abdominal Discomfort
   - Change from baseline in 12-week Bloating

   These 3 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure (22) to control for multiple parameters within this step.

4. The fourth step tested the following 2 secondary parameters:
   - 6/12 Week CSBM +1 Responder
   - 6/12 Week Abdominal Pain Responder

   These 2 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters within this step.

5. The fifth step tested the following single secondary parameter:
   - Change from baseline in 12-week Percent of Abdominal Pain-free Days

   This secondary parameter was tested using a type I error rate of 0.05.

This reviewer's comments on this gatekeeping procedure were

> The sponsor's gatekeeping procedure was not appropriate. The Hochberg procedure is generally not recommended for sequential testing. It is not assumption free. Furthermore, it is known to provide overall α-control for independent and for certain types of positive correlated endpoints. But its properties for other types of dependent endpoints are not fully known. Various simulation experiments indicate that this method generally controls the overall Type 1 error rate for positive correlated endpoints but fails to do so for some negatively correlated endpoints.
>
> The sponsor should use a Bonferroni based gatekeeping procedure to test all endpoints in the primary family and proceed to the secondary family of endpoints only if there has been statistical success in the primary family.

Furthermore, since p-values for most secondary endpoints were very small (<0.001), all secondary endpoints would pass any statistical procedure for controlling the type 1 error for multiplicity.

3.1.1.3.8 Reviewer's Comments on Results of Secondary Efficacy Endpoints

The sponsor's pre-specified analysis for the secondary endpoints was based on a modeling approach (ANCOVA) using all data for each week 1-12. The term "treatment overall" refers to an average treatment effect over the 12 weeks of the study. (b) (4)

3.1.2 Study LIN-MD-31

3.1.2.1 Study Design

This study was a phase 3, randomized, double-blind, placebo-controlled, parallel-group trial of linaclotide administered orally for 12 Weeks followed by a 4-Week randomized withdrawal period in patients with Irritable Bowel Syndrome with Constipation (IBS-C). A total of 118 centers (111 in the United States, 7 in Canada) enrolled patients into the study.

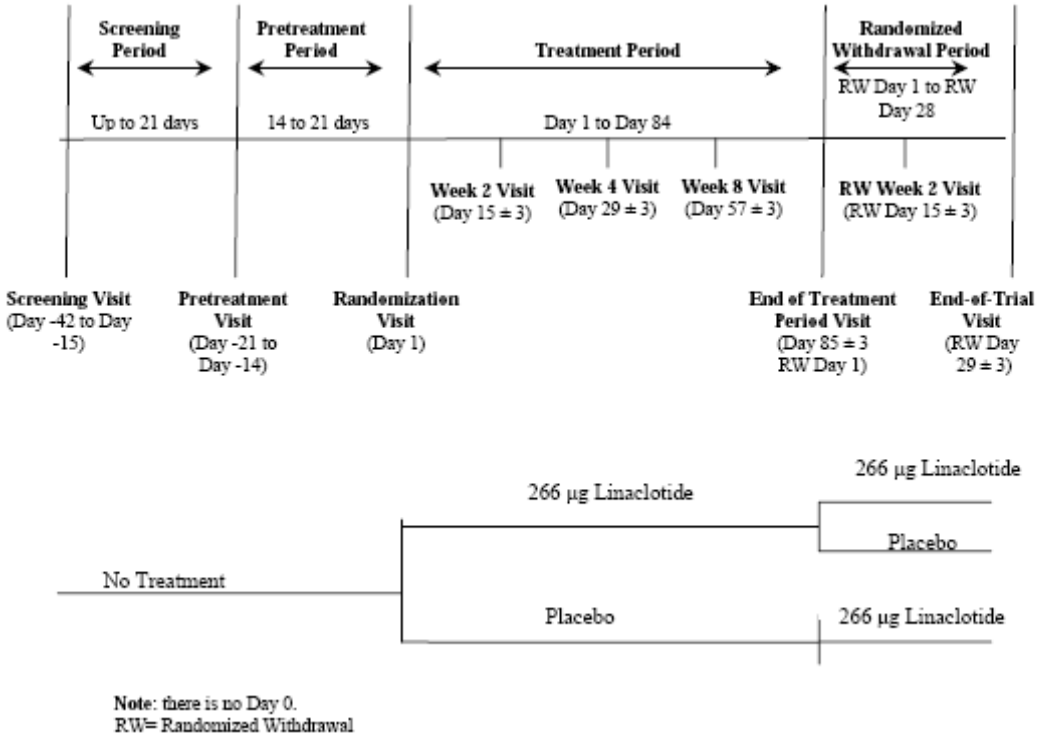
The objective of this trial was to determine the efficacy and safety of linaclotide administered to patients with irritable bowel syndrome with constipation (IBS-C).

This study was designed for comparing a 266 µg/day dose of linaclotide with placebo in patients who met modified Rome II criteria for IBS-C. An interactive voice response system (IVRS) was used by study sites to randomize patients, supply study drug, and record the patient diary information.

As shown in the figure below, the trial consisted of up to 21 days of screening (screening period), 14 to 21 days of pretreatment (pretreatment period), 12 weeks of double-blind treatment (treatment period), and a 4-week double-blind randomized withdrawal (RW) period.

During the screening period the patient's eligibility for entry into the pretreatment period was determined. Any over-the-counter or prescription laxatives, suppositories, or enemas, and any herbal or natural agents used to treat IBS-C were to be discontinued prior to the calendar day before the pretreatment visit (Visit 2). Likewise, non-steroidal anti-inflammatory drugs (NSAIDs), if taken for abdominal pain or discomfort, and any medicines used to treat diarrhea were also to be discontinued prior to the calendar day before the pretreatment visit. Other prohibited medicines (defined in the study protocol) were not to be taken starting from 14 calendar days before the pretreatment visit. During the pretreatment period patients provided qualifying bowel habit, abdominal symptom severity, and rescue medicine information (through the IVRS). At the end of the pretreatment period (Randomization Visit; Visit 3), patients meeting the entry criteria for this trial were randomized to 1 of 2 double-blind treatment groups: 266 µg linaclotide, or placebo (1:1).

Patients continued to provide IVRS diary data throughout the double-blind treatment period. Patients who completed the 12-week treatment period entered the RW period, during which patients who had been treated with linaclotide were re-randomized to either 266 µg linaclotide or placebo (1:1) and patients who had been treated with placebo were allocated to 266 µg linaclotide.



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Linaclotide doses reflect total linaclotide content, rather than total peptide content presented in the protocol. Total linaclotide content doses of 133 µg and 266 µg correspond to total peptide content doses of 150 µg and 300 µg, respectively.

Patients were included if they met the following criteria:

- Males and females aged 18 years and older
- Patient reported abdominal discomfort or pain that had two or more of the following three features for at least 12 weeks, which need not be consecutive, in the 12 months before the Screening Visit (Visit 1) or before starting chronic treatment with tegaserod or lubiprostone:
  (1) Relieved with defecation;
  (2) Onset associated with a change in frequency of stool;
  (3) Onset associated with a change in form (appearance) of stool.
- Patient reported < 3 bowel movements (BMs) per week, with each BM occurring in the absence of laxative/enema/suppository use during the preceding 24 hours and had 1 or more of the following symptoms for at least 12 weeks, which need not be consecutive, in the preceding 12 months:
  (1) Straining during > 25% of BMs;

(2) Lumpy or hard stools during > 25% of BMs; and
(3) Sensation of incomplete evacuation during > 25% of BMs.

- Patient had an average score ≥ 3.0 for abdominal pain at its worst as reported in the IVRS using an 11-point numerical rating scale (NRS) during the 14 calendar days before the start of the treatment period.

In addition, patients had to report an average of < 3 complete spontaneous BMs (CSBMs) per week and 5 or fewer spontaneous BMs (SBMs) per week by the IVRS during the 14 days before the start of the treatment period, and be compliant with IVRS completion by adequately responding to IVRS questions on 10 or more of the 14 days before the start of the treatment period. An SBM was defined as a BM that occurred in the absence of laxative, enema, or suppository use on either the calendar day of the BM or the calendar day before the BM. A CSBM was defined as an SBM that was associated with a sense of complete evacuation.

Patients were excluded for any of the following reasons:
(1) They reported loose (mushy) stools for > 25% of their BMs during the 12 weeks before the Screening Visit;
(2) During the Pretreatment Period, they reported a Bristol Stool Form Scale (BSFS) score of 7 for any SBM or a BSFS score of 6 for more than 1 SBM;
(3) Patient used rescue medicine (bisacodyl tablet or suppository) or any other laxative, suppository, or enema, on the calendar day before or the calendar day of the start of the Treatment Period (i.e., before the Randomization Visit).

The primary efficacy assessments were abdominal pain and BMs that met the criteria for CSBMs, based on the IVRS information.

3.1.2.2 Sponsor's Analysis

Overall, 803 patients were randomized to treatment; two patients were randomized at more than 1 study center but were only counted once A total of 802 patients received double-blind study drug and were included in the Safety Population, and 800 patients had at least 1 post-randomization entry of the primary efficacy assessment and were included in the ITT Population. A total of 647 patients entered the RW period of the study, 645 of whom received at least 1 dose of study drug and were included in the RW Population.

Patient Population
Study LIN-MD-31

Patients screened = 2424			
Screen failures = 466			
Pretreatment failures = 1155			
	Placebo	*Linaclotide*	*Total*
Patients randomized	397	406	803
Safety Population	396	406	802
Intent-to-Treat Population	395	405	800
Entered RW period	335	312	647

RW = randomized withdrawal.
Source: Table 14.1.1 and Table 14.1.2.

Overall, 80.6% of patients completed the double-blind treatment period in the study. A greater percentage of patients treated with linaclotide discontinued from the study than did patients treated with placebo (23.2% vs. 15.6%). This difference was a reflection of the higher percentage of patients treated with linaclotide who discontinued as a result of an AE (7.9% vs. 2.5%; p = 0.0007).

Number (%) of Patients Discontinued From the Study during the Double-Blind Treatment Period—Randomized Population
Study LIN-MD-31

	Placebo (N = 397)	*Linaclotide (N = 406)*	*Total (N = 803)*	*P-value*
	n (%)	n (%)	n (%)	
Completed treatment period	335 (84.4)	312 (76.8)	647 (80.6)	
Discontinued from treatment period	62 (15.6)	94 (23.2)	156 (19.4)	0.0074
Reason for discontinuation				
Adverse event	10 (2.5)	32 (7.9)	42 (5.2)	0.0007
Protocol violation	9 (2.3)	10 (2.5)	19 (2.4)	1.0000
Withdrawal of consent	25 (6.3)	25 (6.2)	50 (6.2)	1.0000
Lost to follow-up	10 (2.5)	17 (4.2)	27 (3.4)	0.2405
Insufficient therapeutic response	4 (1.0)	5 (1.2)	9 (1.1)	1.0000
Other	4 (1.0)	5 (1.2)	9 (1.1)	1.0000

P-values for comparison of linaclotide with placebo used the Fisher exact test.
Source: Table 14.1.3A.

A total of 120 patients had protocol deviations that fell into at least 1 of these 4 classes; among these patients there were 28 class 1 deviations, no class 2 deviations, and 6 class 3 deviations, and 88 class 4 deviations.

3.1.2.2.1 Planned Analysis

The primary efficacy assessments were abdominal pain and BMs that met the criteria for CSBMs, based on the IVRS information.

There were four primary efficacy parameters:

- 9/12 week APC (abdominal pain and CSBM) 3+1 responder
  This patient met the weekly APC 3+1 responder criteria for at least 9 out of the 12 week of the treatment period. A weekly APC 3+1 responder was a patient who had at least 3 CSBMs and an increase of at least 1 CSBM from baseline, and a decrease of at least 30% in the mean abdominal pain score, during a particular week.
- 9/12 week CSBM 3+1 responder
  This patient met the weekly CSBM 3+1 responder criteria for at least 9 out of the 12 weeks of the treatment period. A weekly CSBM 3+1 responder was a patient who had at least 3 CSBMs and an increase of at least 1 CSBM from baseline during a particular week.
- 9/12 week abdominal pain responder
  This patient met the weekly abdominal pain responder criteria for at least 9 out of the 12 weeks of the treatment period. A weekly abdominal pain responder was a patient who had a decrease of at least 30% in the mean abdominal pain score from baseline during a particular week.
- 6/12 week APC +1 responder
  This patient met the weekly APC +1 responder criteria for at least 6 out of the 12 week of the treatment period. A weekly APC +1 responder was a patient who had an increase of at least 1 CSBM from baseline, and a decrease of at least 30% in the mean abdominal pain score, during a particular week.

For each primary efficacy parameter, a patient had to have ≥ 4 complete IVRS calls for a particular treatment period week to be considered a responder for that week.

The secondary efficacy parameters based on the IVRS calls were:

- Change from baseline in 12-week CSBM frequency rate
- Change from baseline in 12-week SBM frequency rate
- Change from baseline in 12-week stool consistency
- Change from baseline in 12-week severity of straining
- Change from baseline in 12-week abdominal pain
- Change from baseline in 12-week abdominal discomfort
- Change from baseline in 12-week bloating
- Change from baseline in 12-week percent of abdominal pain-free days
- 6/12 week CSBM +1 responder (i.e., a patient who had an increase of at least 1 CSBM from baseline per week for 6 of the 12 weeks of treatment)
- 6/12 week abdominal pain responder

The consistency of each BM was assessed by patients using the 7-point ordinal BSFS (1 = separate hard lumps like nuts [difficult to pass]; 2 = sausage shaped but lumpy; 3 = like a sausage but with cracks on surface; 4 = like a sausage or snake, smooth and

soft; 5 = soft blobs with clear-cut edges [passed easily]; 6 = fluffy pieces with ragged edges, a mushy stool; 7 = watery, no solid pieces [entirely liquid]).

Straining is measured on a 5-point ordinal scale where a value of 1 is "not at all" and a value of 5 is "an extreme amount."

Abdominal pain at its worst (in the last 24 hours) is based on an 11-point NRS scale where a value of 0 is "none" and a value of 10 is "very severe."

Abdominal discomfort is based on an 11-point NRS scale where a value of 0 is "none" and a value of 10 is "very severe."

Bloating was based on an 11-point NRS scale where a value of 0 is "none" and a value of 10 is "very severe".

All efficacy analyses during the treatment period were based on the Intent-to-Treat Population, which included all randomized patients who received at least 1 dose of study drug during the double-blind treatment period, and who had at least 1 post-randomization entry of the primary efficacy assessment (i.e., the assessment of abdominal pain at its worst or daily IVRS information that determined whether an SBM was a CSBM).

For the RW period and the combined 16-week treatment + RW periods, efficacy analyses were based on the RW Population, which included all patients who were registered in IVRS to enter the RW period and who received at least 1 dose of study drug during the RW period.

For each of the primary efficacy parameters, the proportion of responders in the linaclotide group was compared to the proportion in the placebo group using the Cochran-Mantel-Haenszel (CMH) test controlling for geographic region. The number and percentage of responders for each treatment group, the difference in responder rates between the linaclotide group and the placebo group, odds ratio, the corresponding confidence intervals, and the two-sided p-values associated with the CMH tests were presented.

All secondary efficacy parameters were based on the 12-week treatment period. The dichotomous secondary efficacy parameters were analyzed using a CMH test controlling for geographic region. All continuous secondary efficacy parameters ("change from baseline") were analyzed using an analysis of covariance (ANCOVA) model with fixed effect terms for treatment group and geographic region and the patient's corresponding baseline value of the parameter as a covariate. Least squares means for each treatment group, difference in least squares means between linaclotide treatment and placebo treatment, associated 2-sided 95% confidence interval for the differences in least squares means, and the corresponding p-value were reported. In addition to inferential and descriptive statistics, results for the secondary efficacy parameters were also displayed graphically by plotting the distribution of responses by treatment group to more fully

characterize the treatment effect. Corresponding weekly summary statistics were also provided.

The overall type I family-wise error rate for testing the primary and secondary efficacy parameters was controlled at the 0.05 significance level using a 5-step serial gatekeeping multiple comparisons procedure.

The overall type I family-wise error rate for testing the primary and secondary efficacy parameters was controlled at the 0.05 significance level using the following 5-step serial gatekeeping multiple comparisons procedure (MCP). Following this MCP, progression to the next step only occurred if all individual null hypotheses within a step were rejected and the previous step(s) were all rejected at the step-specific overall significance level. If all null hypotheses within a step were not rejected, the statistical tests corresponding to all subsequent steps were considered not statistically significant. All hypothesis tests were two-sided.

1. The first step tested the 4 primary efficacy parameters using a fixed sequential testing method. The 4 primary efficacy parameters were each tested at the 0.05 significance level in the following fixed sequence:

    1. 9/12 Week APC 3+1 Responder
    2. 9/12 Week CSBM 3+1 Responder
    3. 9/12 Week Abdominal Pain Responder
    4. 6/12 Week APC +1 Responder

    If a null hypothesis was not rejected (i.e., p-value > 0.05), all subsequent statistical tests were not considered statistically significant.

2. The second step tested the following 4 secondary parameters:
    - Change from baseline in 12-week CSBM Frequency Rate
    - Change from baseline in 12-week SBM Frequency Rate
    - Change from baseline in 12-week Stool Consistency
    - Change from baseline in 12-week Severity of Straining

    These 4 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure (22) to control for multiple parameters within this step.

3. The third step tested the following 3 secondary parameters:
    - Change from baseline in 12-week Abdominal Pain
    - Change from baseline in 12-week Abdominal Discomfort
    - Change from baseline in 12-week Bloating

    These 3 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure (22) to control for multiple parameters within this step.

4. The fourth step tested the following 2 secondary parameters:
   - 6/12 Week CSBM +1 Responder
   - 6/12 Week Abdominal Pain Responder

   These 2 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters within this step.

5. The fifth step tested the following single secondary parameter:
   - Change from baseline in 12-week Percent of Abdominal Pain-free Days

   This secondary parameter was tested using a type I error rate of 0.05.

All confidence intervals were 2-sided 95% confidence intervals.

For this trial, the sample size was planned to be approximately 800 patients, with 400 patients randomized to each of the two treatment groups: 266 µg linaclotide and placebo. This sample size was based on consideration of the overall efficacy results of study MCP-103-202, a 12-week, Phase 2b, double-blind, randomized study in 420 IBS-C patients. However, there are differences between that Phase 2b study and Study LIN-MD-31 that had the potential to impact responder rates, most notably the increased availability of rescue medicine and the modification to the wording, scale, and responder definition of the IVRS daily abdominal pain at its worst assessment. Given the unknown impact of these differences in study design between the Phase 2b study and LIN-MD-31, it was deemed appropriate to have a larger sample size than may be indicated by solely considering the Phase 2b power calculation results. Table below summarizes the overall responder rate estimates for the primary efficacy parameters used in the power and sample size calculations for this trial.

Primary Efficacy Parameters Power Calculations: 266 µg Linaclotide Dose Estimates
Study LIN-MD-31

Primary Efficacy Parameters	*Placebo Estimate*	*266 µg Estimate*	*Nominal Power*	*Multiplicity Adjusted Power*
1) 9/12 Week APC 3+1 Responder	10.0%	24.0%	> 99%	> 99%
2) 9/12 Week CSBM 3+1 Responder	12.5%	28.0%	> 99%	> 99%
3) 9/12 Week Abdominal Pain Responder	25.0%	45.3%	> 99%	> 99%
4) 6/12 Week APC +1 Responder	27.5%	49.3%	> 99%	> 99%

Note: The primary efficacy parameter rate estimates for placebo and linaclotide 266 µg are based on Phase 2b responder rates from the placebo and the linaclotide 266 µg dose groups, respectively, incorporating the 4-complete-IVRS-call criteria. For each parameter, the nominal power is the probability of the p-value for the treatment group comparison being < 0.05. The multiplicity adjusted power estimates are based on 100,000 computer simulations that incorporate the fixed sequential testing method described in Section 9.7.1.5.3.

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With 400 randomized patients per treatment group arm, and using the responder

estimates from Phase 2b as presented in Table above, the adjustments for multiplicity and a two-sample Chi-square two-sided test at the 5% significance level, the power to reject all 4 primary efficacy parameters is greater than 99% (equivalent to rejecting the 6/12 week APC +1 responder parameter due to the fixed sequential testing procedure methodology). The nominal power for each of the individual primary efficacy parameters is also provided in Table above.

Based on more conservative estimates for linaclotide responder rate (i.e., by pooling all linaclotide doses rather than just using the linaclotide dose of 266 μg from the Phase 2b IBS-C study), the power estimates for the 4 primary efficacy parameters with 400 randomized patients per treatment group arm are provided in Table above. Overall, the power to reject all 4 primary efficacy parameters is > 85%

3.1.2.2.2 Treatment Group Comparability

The summary of results of comparability of treatment groups at baseline for all randomized patients is given in Appendix Tables 3 and 4.

As seen from Appendix Table 3, demographics and baseline characteristics were comparable between treatment groups.

As seen from Appendix Table 4, the patients treated with linaclotide had lower mean BSFS scores and higher mean straining scores at baseline relative to patients treated with placebo.

The most commonly used concomitant medications (≥ 5%) were generally similar between the treatment groups, and were typical for this patient population.

In the safety population, the median age of subjects was 44 years. Most subjects were white (77%), and the majority were female (91%).

Compliance rates (patients with ≥ 80% complete calls) were 73.2% for patients treated with placebo and 71.1% for patients treated with linaclotide.

3.1.2.2.3 Sponsor's Analysis of Primary Efficacy Variable

The primary endpoint was the number of patients who were 9/12 week APC 3+1 responders, defined as patients who were APC 3+1 responders for at least 9 of the 12 weeks of the treatment period. For each week in the treatment period, a weekly APC 3+1 responder was a patient who had at least 3 CSBMs for the week and an increase of at least 1 CSBM from baseline for that week, and also had a decrease of at least 30% in the mean abdominal pain score for that week.

The result from analysis of 9/12 week abdominal pain and CSBM (APC) 3+1 responders in the ITT population is given below.

Primary Efficacy Analysis: 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders—ITT Population
Study LIN-MD-31

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	Placebo (N = 395)	*Linaclotide (N= 405)*	*Statistics*
Responder, n (%)	20 (5.1)	49 (12.1)	
Nonresponder, n (%)	375 (94.9)	356 (87.9)	
Difference in responder rate (linaclotide – placebo)	—	—	7.0
Odds ratio (95% CI)	—	—	2.60 (1.51, 4.47)
P-value	—	—	0.0004

A 9/12 week APC 3+1 responder was a patient who met the weekly APC 3+1 responder criteria for at least 9 of the 12 weeks of the double-blind treatment period.

Odds ratios, 95% CI and p-values were obtained from the Cochran-Mantel-Haenszel method controlling for geographic region.

The p-value met the criterion for statistical significance based on the multiple comparison procedure.

CI = confidence interval; CSBM = complete spontaneous bowel movement; ITT = intent-to-treat; N = population size; n = number of responders within a group.

Source: Table 14.4.1.1.

As seen from the table above, the percentage of responders in the linaclotide treatment group was over twice that in the placebo group (12.1% vs. 5.1%).

3.1.2.2.4 Sponsor's Analysis of Other Three Primary Efficacy Variable

The next two primary efficacy parameters (9/12 week CSBM 3+1 responders and 9/12 week abdominal pain responders) are the separate components of the first primary efficacy parameter. 9/12 week CSBM 3+1 responders was defined as patients who were CSBM 3+1 responders for at least 9 of the 12 weeks of the treatment period, and 9/12 week abdominal pain responders was defined as patients who were abdominal pain responders for at least 9/12 week of the treatment period.

The results from analysis of 9/12 week abdominal pain and CSBM (APC) 3+1 and 9/12 abdominal pain responder endpoints in the ITT population are given below.

Primary Efficacy Analysis: 9/12 Week CSBM 3+1 Responders
ITT Population
Study LIN-MD-31

	Placebo (N = 395)	*Linaclotide (N = 405)*	*Statistics*
Responder, n (%)	25 (6.3)	79 (19.5)	
Nonresponder, n (%)	370 (93.7)	326 (80.5)	
Difference in responder rate (linaclotide – placebo)	—	—	13.2
Odds ratio (95% CI)	—	—	3.65 (2.26, 5.88)
P-value	—	—	< 0.0001

A 9/12 week CSBM 3+1 responder was a patient who met the weekly CSBM 3+1 responder criteria for at least 9 of the 12 weeks of the double-blind treatment period.
Odds ratios, 95% CI and p-values were obtained from the Cochran-Mantel-Haenszel method controlling for geographic region.
The p-value met the criterion for statistical significance based on the multiple comparison procedure.
CI = confidence interval; CSBM = complete spontaneous bowel movement; ITT = intent-to-treat; N = population size; n = number of responders within a group.
Source: Table 14.4.1.2.

Primary Efficacy Analysis: 9/12 Week Abdominal Pain Responders
ITT Population
Study LIN-MD-31

	Placebo (N = 395)	*Linaclotide (N= 405)*	*Statistics*
Responder, n (%)	107 (27.1)	139 (34.3)	
Nonresponder, n (%)	288 (72.9)	266 (65.7)	
Difference in responder rate (linaclotide – placebo)	—	—	7.2
Odds ratio (95% CI)	—	—	1.41 (1.04, 1.91)
P-value	—	—	0.0262

A 9/12 week abdominal pain responder was a patient who met the weekly abdominal pain responder criteria for at least 9 of the 12 weeks of the double-blind treatment period.
Odds ratios, 95% CI and p-values were obtained from the Cochran-Mantel-Haenszel method controlling for geographic region.
The p-value met the criterion for statistical significance based on the multiple comparison procedure.
CI = confidence interval; ITT = intent-to-treat; N = population size; n = number of responders within a group.
Source: Table 14.4.1.3.

As seen from tables above, the percentage of 9/12 Week CSBM 3+1 responders in the linaclotide treatment group was statistically significantly higher than that in the placebo group). The percentage of 9/12 week abdominal pain responders in the linaclotide treatment group was also statistically significantly higher than that in the placebo group.

The fourth primary efficacy endpoint, 6/12 week APC +1 responder was defined as this patient met the weekly APC +1 responder criteria for at least 6 out of the 12 week of the treatment period. A weekly APC +1 responder was a patient who had an increase of at least 1 CSBM from baseline, and a decrease of at least 30% in the mean abdominal pain score, during a particular week.

The results from analysis of 6/12 week abdominal pain and CSBM (APC) +1 endpoint in the ITT population is given below.

Primary Efficacy Analysis: 6/12 Week APC+1 Responders - ITT Population Study LIN-MD-31

	Placebo (N = 395)	*Linaclotide (N = 405)*	*Statistics*
Responder, n (%)	83 (21.0)	136 (33.6)	
Nonresponder, n (%)	312 (79.0)	269 (66.4)	
Difference in responder rate (linaclotide – placebo)	—	—	12.6
Odds ratio (95% CI)	—	—	1.93 (1.40, 2.66)
P-value	—	—	< 0.0001

A 6/12 week APC +1 responder was a patient who met the weekly APC +1 responder criteria for at least 6 of the 12 weeks of the double-blind treatment period.

Odds ratios, 95% CI and p-values were obtained from the Cochran-Mantel-Haenszel method controlling for geographic region.

The p-value met the criterion for statistical significance based on the multiple comparison procedure.

CI = confidence interval; ITT = intent-to-treat; N = population size; n = number of responders within a group.

Source: Table 14.4.1.4.

As seen from the table above, the percentage of 6/12 week APC +1 responders was 33.6% compared with 21.0% in the placebo group ($p < 0.0001$).

3.1.2.2.5 Sponsor's Analyses of Secondary Variables

The secondary efficacy parameters based on the IVRS calls were:

- Change from baseline in 12-week CSBM frequency rate
- Change from baseline in 12-week SBM frequency rate
- Change from baseline in 12-week stool consistency
- Change from baseline in 12-week severity of straining
- Change from baseline in 12-week abdominal pain
- Change from baseline in 12-week abdominal discomfort
- Change from baseline in 12-week bloating
- Change from baseline in 12-week percent of abdominal pain-free days
- 6/12 week CSBM +1 responder (i.e., a patient who had an increase of at least 1 CSBM from baseline per week for 6 of the 12 weeks of treatment)
- 6/12 week abdominal pain responder

3.1.2.2.5.1Change from Baseline in 12-week CSBM Frequency Rate

Summary of results of analysis of the change from baseline in 12-week CSBM frequency rate (i.e., weekly CSBM frequency rate over the 12-week treatment) is given below.

12-Week CSBM Frequency Rate – ITT Population
Study LIN-MD-31

Visit	*Statistic*	*Placebo (N = 395)*	*Linaclotide (N = 405)*
Baseline	Mean	0.238	0.203
	SD	0.505	0.457
	SEM	0.025	0.023
	Median	0.000	0.000
	Min, max	0.00, 2.90	0.00, 2.43
	n	395	405
Treatment overall	Mean	1.040	2.568
	SD	1.413	3.088
	SEM	0.071	0.153
	Median	0.335	1.490
	Min, max	0.00, 8.62	0.00, 16.54
	n	395	405
ANCOVA results	LSMC from baseline (SE)	0.705 (0.128)	2.272 (0.127)
	LSMD (95% CI)	—	1.568 (1.241, 1.895)
	P-value[a]	—	< 0.0001

a P-values are based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate.

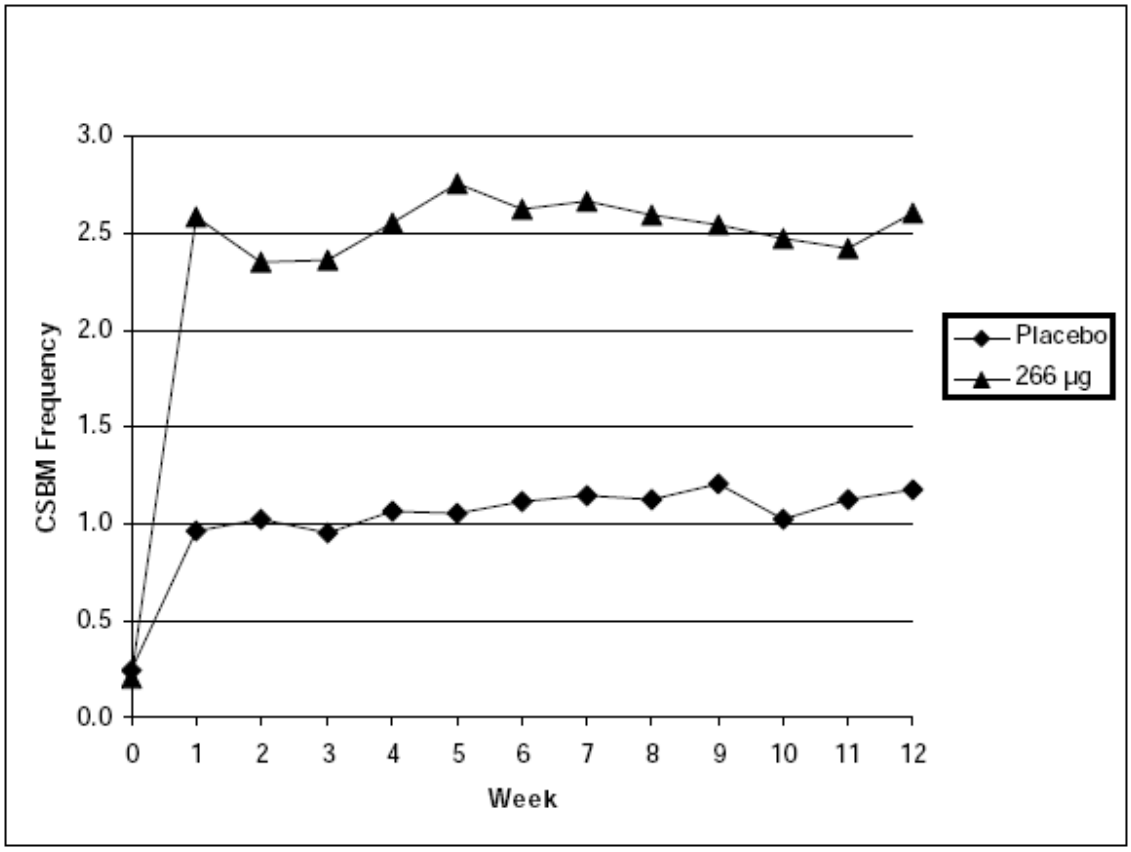
ANCOVA = analysis of covariance; CI = confidence interval; CSBM = complete spontaneous bowel movement; ITT = intent-to-treat; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Source: Table 14.4.2.1A.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean CSBM frequency rate during the treatment period is plotted by week and is given below.

Mean CSBM Rate during Each Week of Treatment Period (OC) – ITT
Study LIN-MD-31



Weekly p-values < 0.0001 for all linaclotide measurements versus placebo; comparisons were based on an ANCOVA change from baseline model with treatment group and geographic region factors and baseline value as covariate.
ANCOVA = analysis of covariance; CSBM = complete spontaneous bowel movement; ITT = intent-to-treat; OC = observed cases.

Source: Table 14.4.2.1B.

Copied from Figure 11.4.1.3.1-1

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.1.2.5.2 Change from Baseline in 12-week SBM Frequency Rate

Summary of results of analysis of the change from baseline in 12-week SBM frequency rate (i.e., weekly SBM frequency rate over the 12-week treatment) is given below.

12-Week SBM Frequency Rate – ITT Population
Study LIN-MD-31

Visit	*Statistic*	*Placebo (N = 395)*	*Linaclotide (N = 405)*
Baseline	Mean	1.897	1.935
	SD	1.399	1.378
	SEM	0.070	0.068
	Median	1.461	1.920
	Min, max	0.00, 6.78	0.00, 6.26
	n	395	405
Treatment overall	Mean	3.174	5.977
	SD	2.222	4.382
	SEM	0.112	0.218
	Median	3.053	5.480
	Min, max	0.00, 12.19	0.00, 40.86
	n	395	405
ANCOVA results	LSMC from baseline (SE)	1.130 (0.177)	3.898 (0.176)
	LSMD (95% CI)	—	2.769 (2.315, 3.223)
	P-value[a]	—	< 0.0001

a P-values are based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate.

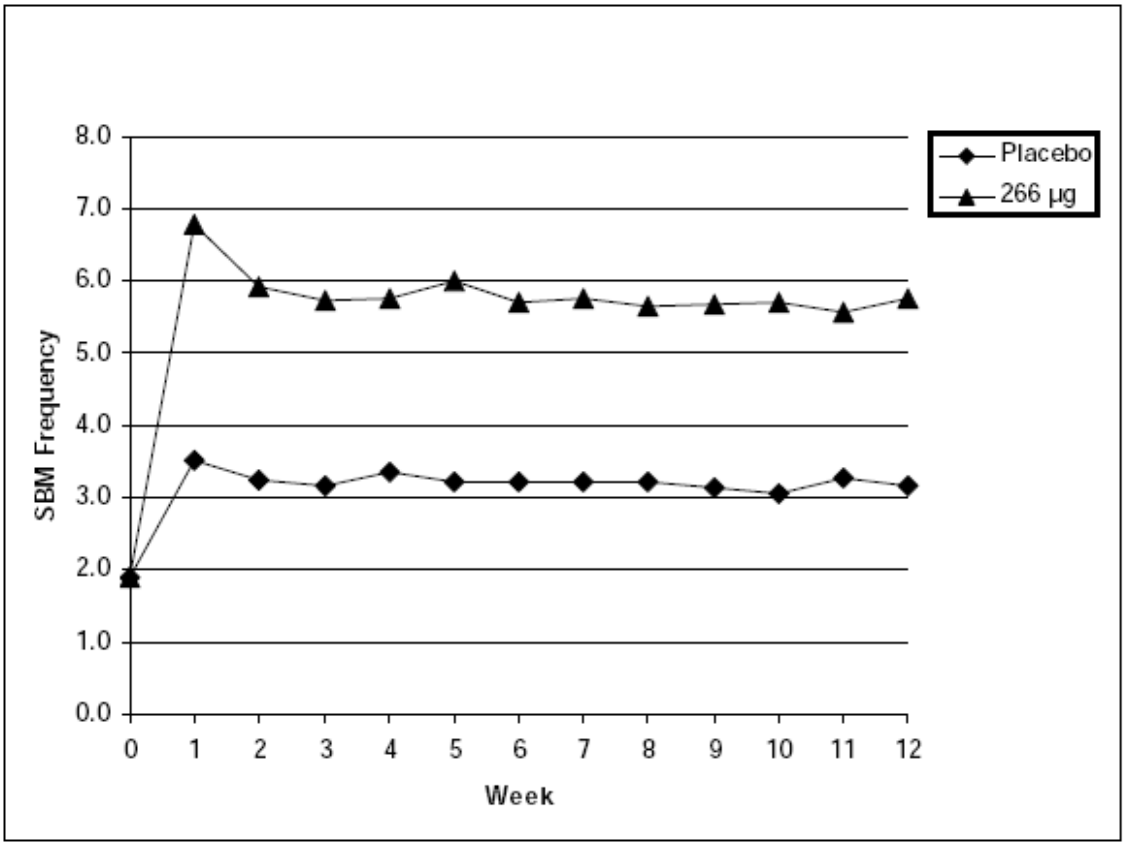
ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point; SBM = spontaneous bowel movement.

Source: Table 14.4.2.2A.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean SBM frequency rate during the treatment period is plotted by week and is given below.

Mean SBM Rate during Each Week of Treatment Period (OC) – ITT
Study LIN-MD-31



Weekly p-values < 0.0001 for all linaclotide measurements versus placebo; comparisons were based on an ANCOVA change from baseline model with treatment group and geographic region factors and baseline value as covariate.
ANCOVA = analysis of covariance; ITT = intent-to-treat; OC = observed cases; SBM = spontaneous bowel movement.
Source: Table 14.4.2.2B

Copied from Figure 11.4.1.3.2-1

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.2.2.5.3 Change from Baseline in 12-week Stool Consistency

Summary of results of analysis of the change from baseline in 12-week stool consistency is given below.

12-Week Stool Consistency -ITT Population
Study LIN-MD-31

Visit	*Statistic*	*Placebo (N = 395)*	*Linaclotide (N = 405)*
Baseline	Mean	2.395	2.260
	SD	1.026	0.994
	SEM	0.056	0.053
	Median	2.200	2.200
	Min, max	1.00, 6.00	1.00, 6.00
	n	338	355
Treatment overall	Mean	3.088	4.454
	SD	0.955	1.238
	SEM	0.052	0.066
	Median	3.148	4.543
	Min, max	1.00, 6.63	1.00, 6.90
	n	338	355
ANCOVA results	LSMC from baseline (SE)	0.662 (0.061)	2.071 (0.060)
	LSMD (95% CI)	—	1.409 (1.253, 1.565)
	P-value[a]	—	< 0.0001

a P-values are based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate.

ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

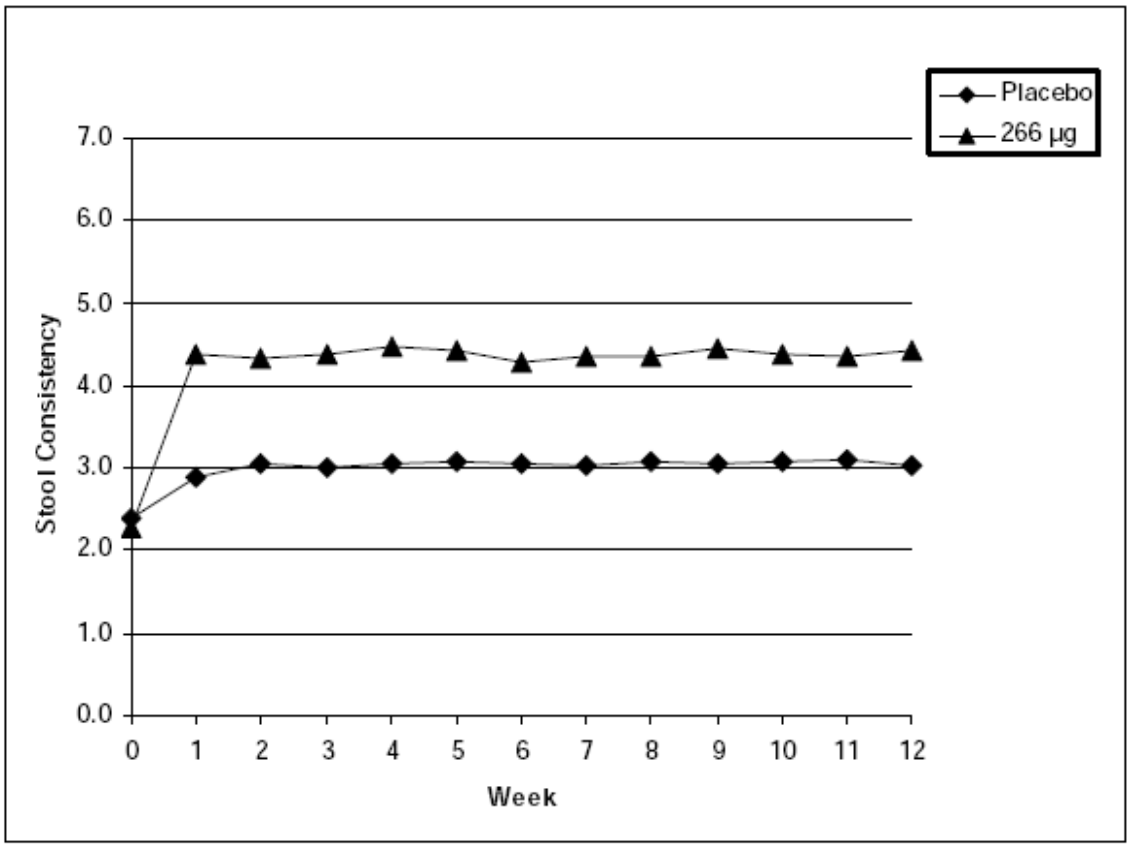
Source: Table 14.4.2.3A.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean stool consistency during the treatment period is plotted by week and is given below.

Mean Stool Consistency during Each Week of Treatment Period (OC) – ITT Population
Study LIN-MD-31

Cop



Weekly p-values < 0.0001 for all linaclotide measurements versus placebo; comparisons were based on an ANCOVA change from baseline model with treatment group and geographic region factors and baseline value as covariate.
ANCOVA = analysis of covariance; BSFS = Bristol Stool Form Scale; ITT = intent-to-treat; OC =observed cases.
Source: Table 14.4.2.3B

Copied from Figure 11.4.1.3.3-1

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.2.2.5.4 Change from Baseline in 12-week Severity of Straining

Summary of results of analysis of the change from baseline in 12-week severity of straining is given below.

12-Week Severity of Straining -ITT Population
Study LIN-MD-31

Visit	*Statistic*	*Placebo (N = 395)*	*Linaclotide (N = 405)*
Baseline	Mean	3.449	3.579
	SD	0.790	0.756
	SEM	0.043	0.040
	Median	3.500	3.500
	Min, max	1.00, 5.00	1.00, 5.00
	n	338	355
Treatment overall	Mean	2.779	2.164
	SD	0.747	0.797
	SEM	0.041	0.042
	Median	2.820	2.082
	Min, max	1.00, 4.91	1.00, 5.00
	n	338	355
ANCOVA results	LSMC from baseline (SE)	–0.651 (0.042)	–1.306 (0.042)
	LSMD (95% CI)	—	–0.655 (–0.763, –0.546)
	P-value[a]	—	< 0.0001

a P-values are based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate.

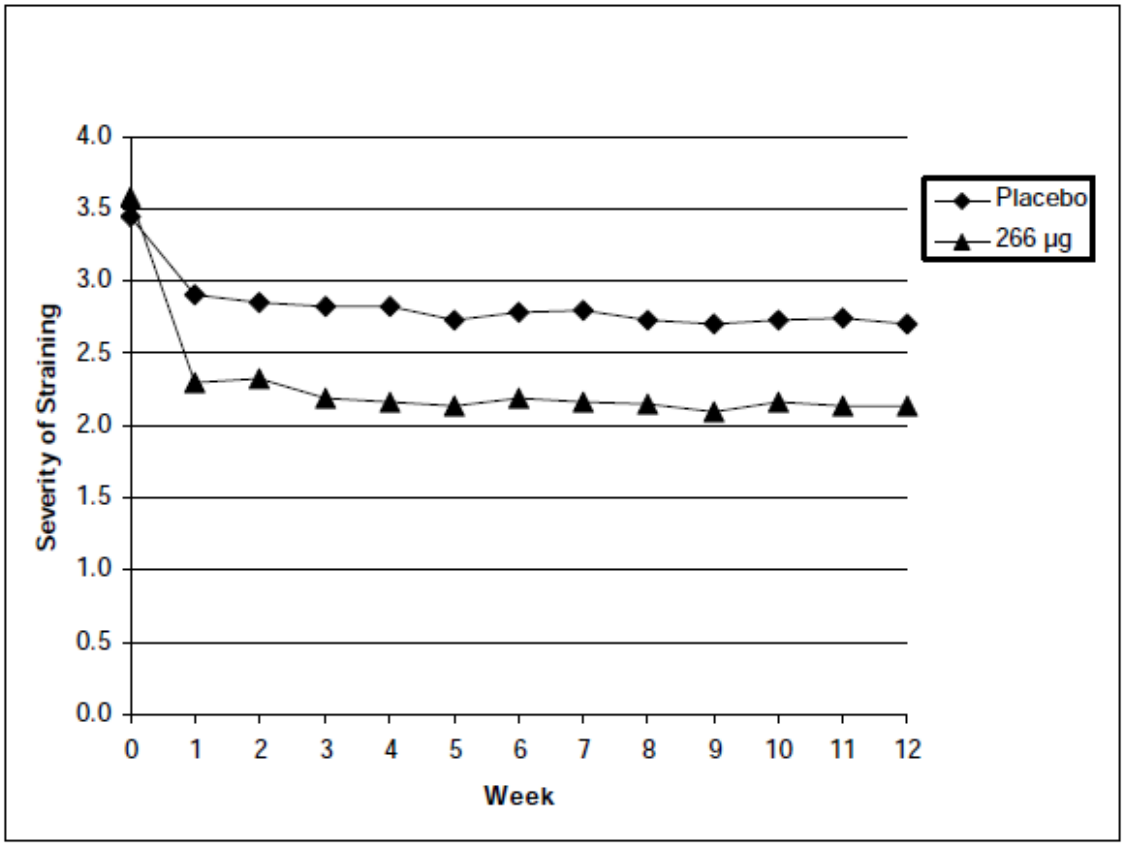
ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Source: Table 14.4.2.4A.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean severity of straining stool consistency during the treatment period is plotted by week and is given below.

Change from Baseline in Mean Severity of Straining During Each Week of Treatment Period (OC)
ITT Population
Study LIN-MD-31



Weekly p-values < 0.0001 for all linaclotide measurements versus placebo; comparisons were based on an ANCOVA change from baseline model with treatment group and geographic region factors and baseline value as covariate.
ANCOVA = analysis of covariance; ITT = intent-to-treat; OC =observed cases.
Source: Table 14.4.2.4B

Copied from Figure 11.4.1.3.4-1

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.2.2.5.5 Change from Baseline in 12-week Abdominal Pain

Summary of results of analysis of the change from baseline in 12-week abdominal pain is given below.

12-Week Abdominal Pain -ITT Population
Study LIN-MD-31

Visit	*Statistic*	*Placebo (N = 395)*	*Linaclotide (N = 405)*
Baseline	Mean	5.633	5.656
	SD	1.707	1.648
	SEM	0.086	0.082
	Median	5.429	5.500
	Min, max	2.79, 10.00	2.93, 10.00
	n	395	405
Treatment overall	Mean	4.377	3.653
	SD	2.194	2.134
	SEM	0.110	0.106
	Median	4.145	3.329
	Min, max	0.13, 9.87	0.00, 10.00
	n	395	405
ANCOVA results	LSMC from baseline (SE)	–1.129 (0.094)	–1.869 (0.093)
	LSMD (95% CI)	—	–0.740 (–0.981, –0.499)
	P-value[a]	—	< 0.0001

a P-values are based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate.

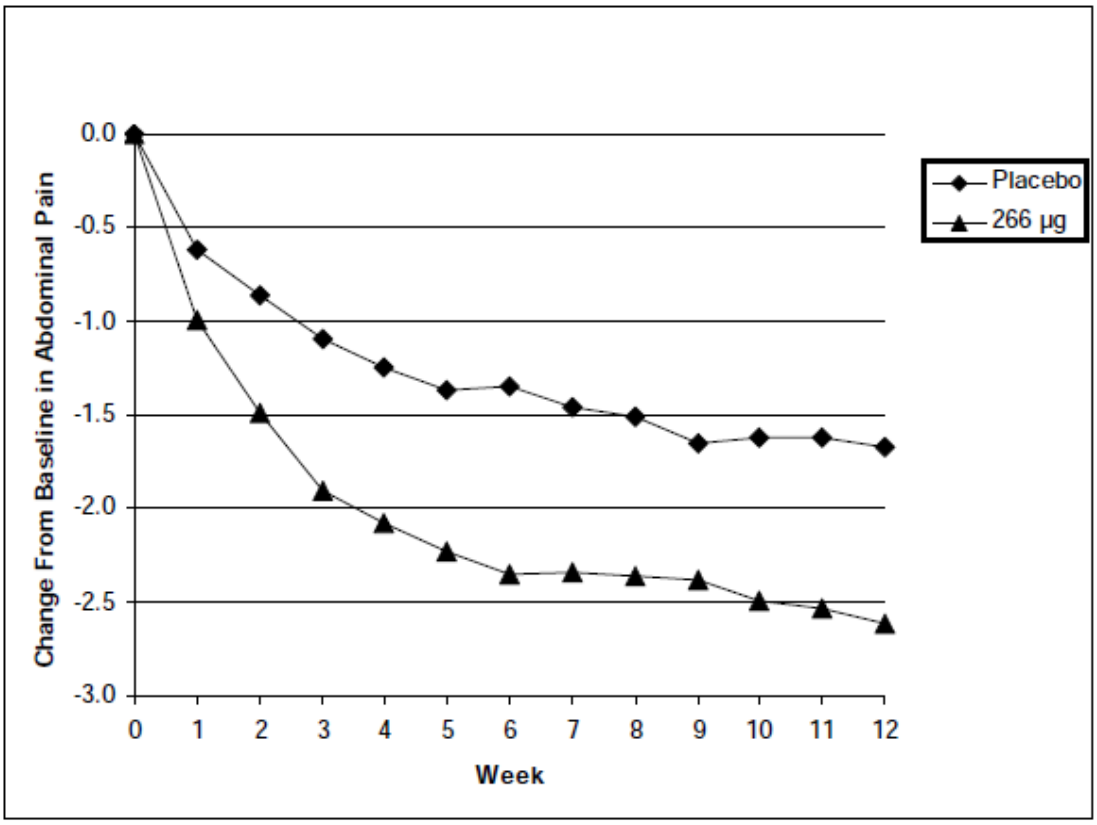
ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Source: Table 14.4.2.5A.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean abdominal pain during the treatment period is plotted by week and is given below.

Change from Baseline in Mean Abdominal Pain during Each Week of Treatment Period (OC) – ITT Population
Study LIN-MD-31



Weekly p-values < 0.0001 for all linaclotide measurements versus placebo except Week 1 (p = 0.0003); comparisons were based on an ANCOVA change from baseline model with treatment group and geographic region factors and baseline value as covariate.

ANCOVA = analysis of covariance; ITT = intent-to-treat; OC = observed cases.

Source: Table 14.4.2.5B.

Copied from Figure 11.4.1.3.5-1

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.2.2.5.6 Change from Baseline in 12-week Abdominal Discomfort

Summary of results of analysis of the change from baseline in 12-week abdominal discomfort is given below.

12-Week Abdominal Discomfort -ITT Population
Study LIN-MD-31

Visit	*Statistic*	*Placebo (N = 395)*	*Linaclotide (N = 405)*
Baseline	Mean	6.041	6.170
	SD	1.672	1.600
	SEM	0.084	0.079
	Median	5.929	6.133
	Min, max	2.33, 10.00	2.85, 10.00
	n	395	405
Treatment overall	Mean	4.721	4.070
	SD	2.145	2.146
	SEM	0.108	0.107
	Median	4.584	3.787
	Min, max	0.38, 10.00	0.13, 10.00
	n	395	405
ANCOVA results	LSMC from baseline (SE)	–1.211 (0.097)	–1.953 (0.096)
	LSMD (95% CI)	—	–0.742 (–0.990, –0.494)
	P-value[a]	—	< 0.0001

a P-values are based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate.

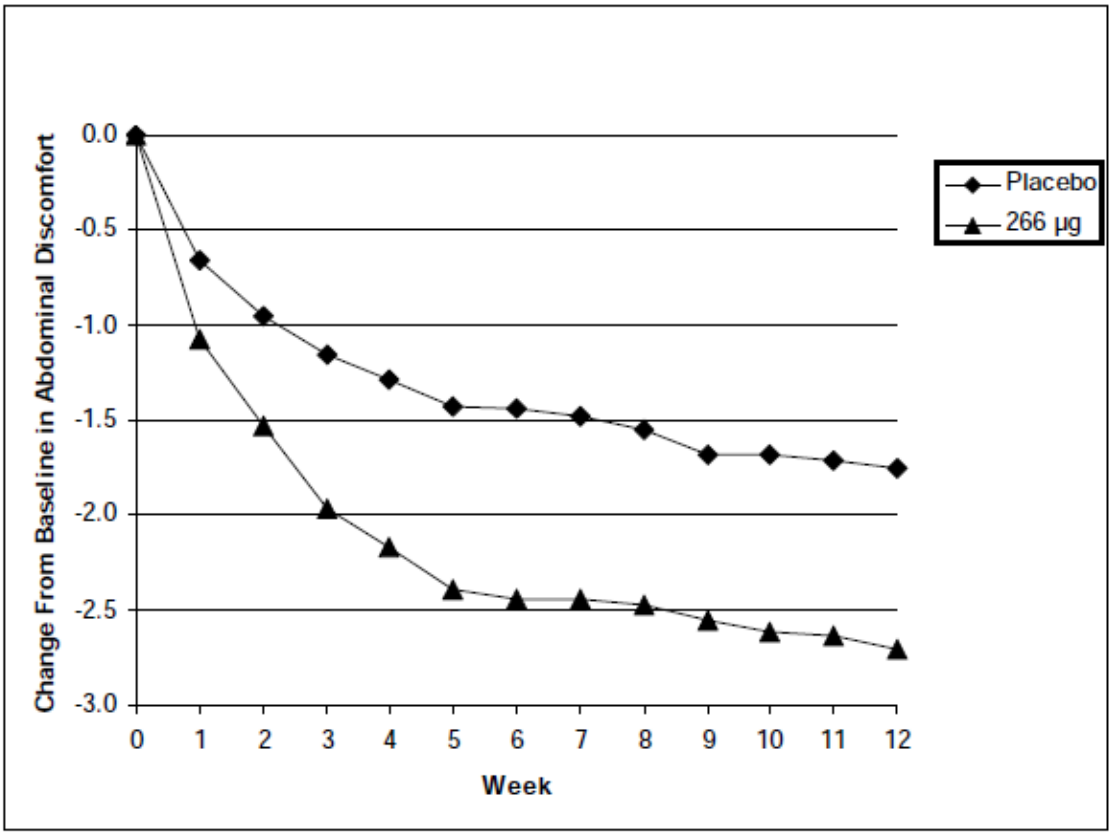
ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Source: Table 14.4.2.6A.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean abdominal discomfort during the treatment period is plotted by week and is given below.

Change from Baseline in Mean Abdominal Discomfort during Each Week of Treatment Period (OC) – ITT Population
Study LIN-MD-31



Weekly p-values ≤ 0.0001 for all linaclotide measurements versus placebo; comparisons were based on an ANCOVA change from baseline model with treatment group and geographic region factors and baseline value as covariate.
ANCOVA = analysis of covariance; ITT = intent-to-treat; OC = observed cases.
Source: Table 14.4.2.6B.
Copied from Figure 11.4.1.3.6-1.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.2.2.5.7 Change from Baseline in 12-week Bloating

Summary of results of analysis of the change from baseline in 12-week bloating is given below.

12-Week Bloating -ITT Population
Study LIN-MD-31

Visit	*Statistic*	*Placebo (N = 393)*	*Linaclotide (N= 405)*
Baseline	Mean	6.496	6.712
	SD	1.890	1.771
	SEM	0.095	0.088
	Median	6.400	6.929
	Min, max	0.00, 10.00	0.36, 10.00
	n	395	405
Treatment overall	Mean	5.306	4.623
	SD	2.276	2.335
	SEM	0.114	0.116
	Median	5.078	4.465
	Min, max	0.00, 10.00	0.15, 10.00
	n	395	405
ANCOVA results	LSMC from baseline (SE)	–1.100 (0.100)	–1.944 (0.099)
	LSMD (95% CI)	—	–0.844 (–1.101, –0.587)
	P-value[a]	—	< 0.0001

a P-values are based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate.

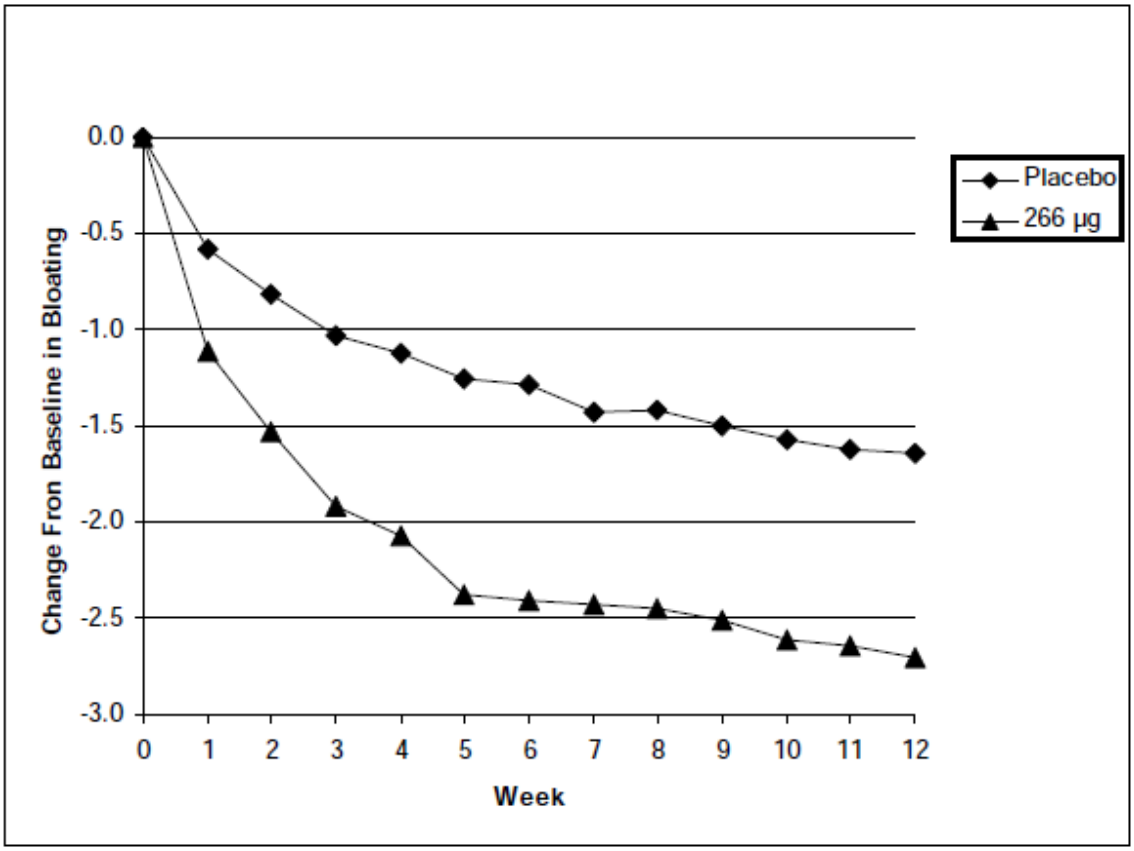
ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Source: Table 14.4.2.7A.

As seen from the table above, the difference between treatment groups was statistically significant.

Mean bloating during the treatment period is plotted by week and is given below.

Change from Baseline in Mean Bloating during Each Week of Treatment Period (OC) – ITT Population
Study LIN-MD-31



Weekly p-values ≤ 0.0001 for all linaclotide measurements versus placebo; comparisons were based on an ANCOVA change from baseline model with treatment group and geographic region factors and baseline value as covariate.
ANCOVA = analysis of covariance; ITT = intent-to-treat; OC = observed cases.
Source: Table 14.4.2.7B.
Copied from Figure 11.4.1.3.7-1.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.2.2.5.8 Change from Baseline in 12-week Percent of Abdominal Pain-free Days

Summary of results of analysis of the change from baseline in 12-week bloating is given below.

12-Week Percent of Abdominal Pain-free Days -ITT Population
Study LIN-MD-31

Visit	*Statistic*	*Placebo (N = 395)*	*Linaclotide (N = 405)*
Baseline	Mean	1.69	2.06
	SD	6.00	6.48
	SEM	0.30	0.32
	Median	0.000	0.000
	Q1, Q3	0.00, 0.00	0.00, 0.00
	Min, max	0.00, 46.7	0.00, 42.9
	n	395	405
Treatment overall	Mean	6.99	11.88
	SD	17.26	23.39
	SEM	0.87	1.16
	Median	0.000	0.000
	Q1, Q3	0.00, 2.70	0.00, 10.00
	Min, max	0.00, 92.3	0.00, 100.0
	n	395	405
Change from Baseline	Mean	5.31	9.81
	SD	15.74	21.75
	SEM	0.79	1.08
	Median	0.00	0.00
	Q1, Q3	0.00, 1.22	0.00, 7.35
	Min, max	–33.8, 85.7	–33.3, 92.6
	n	395	405
	P-value[a]	——	0.0014

a P-values are based on a comparison of linaclotide versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate, using rank-transformed normal scores.

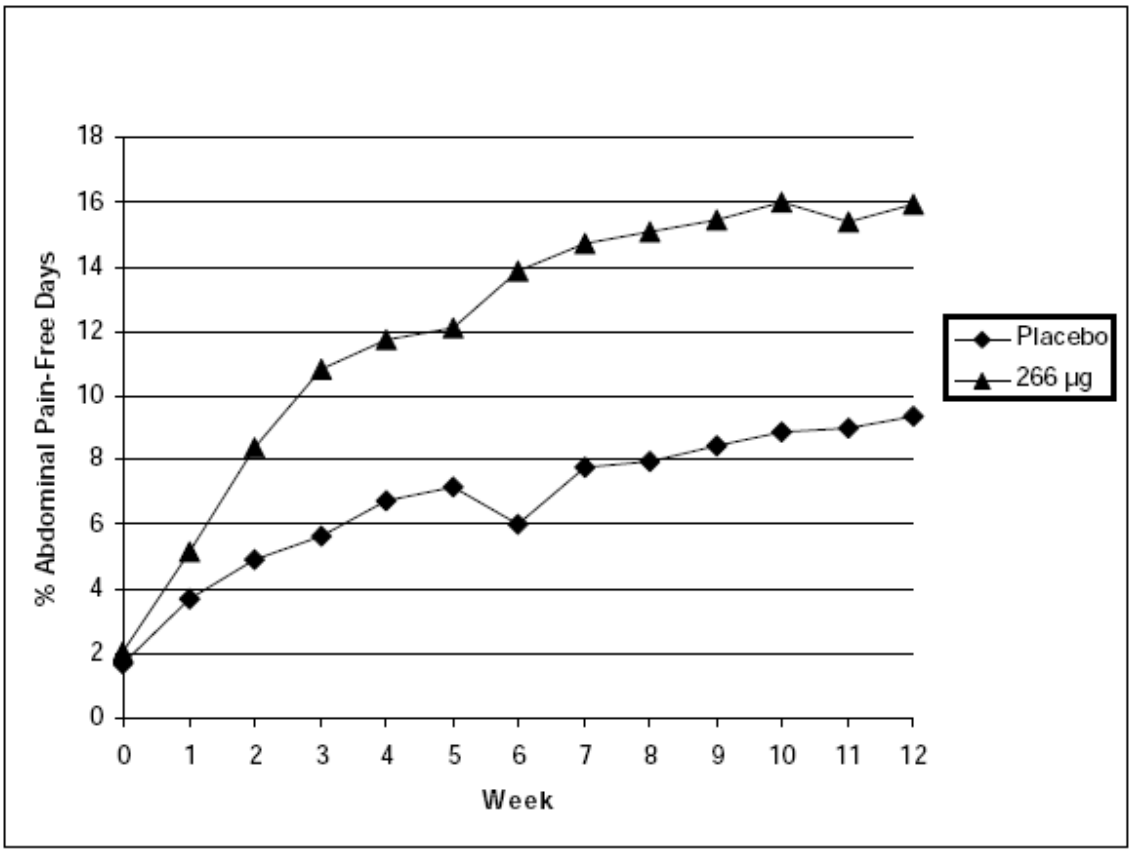
ANCOVA = analysis of covariance; ITT = intent-to-treat; max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point; Q1 = 25th percentile; Q3 = 75th percentile.

Source: Table 14.4.2.10A.

Copied from Figure

Mean percentage of abdominal pain-free days during the treatment period is plotted by week and is given below.

Mean Percent of Abdominal Pain-free During Each Week of Treatment Period (OC) – ITT Population
Study LIN-MD-31



Weekly p-values for weeks 1 and 2 were > 0.05 and weekly p-values for all other weeks were < 0.025 for all linaclotide measurements versus placebo; comparisons were based on an ANCOVA of rank-transformed normal scores of the change from baseline in percent of abdominal pain-free days. The ANCOVA model had factors for treatment group and geographic region and rank-transformed normal scores of the baseline values as covariate.

ANCOVA = analysis of covariance; ITT = intent-to-treat; OC = observed cases.

Source: Table 14.4.2.10B.

Copied from Figure 11.4.1.3.10-1.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 3 and was sustained over the following weeks of the 12-week treatment period.

3.1.2.2.5.9 6/12 week CSBM +1 Responder

This secondary efficacy endpoint was the number of patients who were 6/12-week CSBM +1 responders, defined as patients who were CSBM +1 responders for at least 6 of the 12 weeks of the treatment period. This is a component of the fourth primary efficacy parameter (6/12 week APC +1 responder). For each week in the treatment period, a weekly CSBM +1 responder was a patient who had at an increase of at least 1 CSBM from baseline for that week.

Summary of the results of analysis of 6/12 week CSBM +1 responder is given below.

Secondary Efficacy Analysis: 6/12 Week CSBM + 1 Responders – ITT
Study LIN-MD-31

	Placebo (N = 395)	*Linaclotide (N = 405)*
Responder, n (%)	117 (29.6)	197 (48.6)
Nonresponder, n (%)	278 (70.4)	208 (51.4)
Difference in responder rate (linaclotide – placebo)	—	19.0
Odds ratio (95% CI)	—	2.28 (1.70, 3.06)
P-value	—	< 0.0001

A 6/12 week CSBM +1 responder was a patient who met the weekly CSBM +1 responder criteria for at least 6 of the 12 weeks of the double-blind treatment period.

Odds ratios, 95% CI and p-values were obtained from the Mantel-Haenszel method controlling for geographic region.

P-value met the criterion for statistical significance based on the multiple comparison procedure.

CI = confidence interval; CSBM = complete spontaneous bowel movement; ITT = intent-to-treat; N = population size; n = number of responders within a group.

Source: Table 14.4.2.8.

As seen from the table above, the percentage of responders in the linaclotide treatment group was significantly greater than that in the placebo group.

3.1.2.2.5.10 6/12 Week Abdominal Pain Responder

This secondary efficacy endpoint was the number of patients who were 6/12-week abdominal pain responders, defined as patients who were abdominal pain responders for at least 6 of the 12 weeks of the treatment period. This is a component of the fourth primary efficacy parameter (6/12 week APC +1 responder). For each week in the treatment period, a weekly abdominal pain responder was a patient who had at a decrease of at least 30% in abdominal pain score from baseline for that week.

Summary of the results of analysis of 6/12 week CSBM +1 responder is given below.

Secondary Efficacy Analysis: 6/12 Week Abdominal Pain Responders – ITT Study LIN-MD-31

	Placebo (N = 393)	*Linaclotide (N = 405)*
Responder, n (%)	148 (37.5)	203 (50.1)
Nonresponder, n (%)	247 (62.5)	202 (49.9)
Difference in responder rate (linaclotide – placebo)	—	12.7
Odds ratio (95% CI)	—	1.69 (1.27, 2.24)
P-value	—	0.0003

A 6/12 week abdominal pain responder was a patient who met the weekly abdominal pain responder criteria for at least 6 of the 12 weeks of the double-blind treatment period.

Odds ratios, 95% CI and p-values were obtained from the Mantel-Haenszel method controlling for geographic region.

P-value met the criterion for statistical significance based on the multiple comparison procedure.

CI = confidence interval; CSBM = complete spontaneous bowel movement; ITT = intent-to-treat; N = population size; n = number of responders within a group.

Source: Table 14.4.2.9.

As seen from the table above, percentage of responders in the linaclotide treatment group was significantly greater than that in the placebo group.

3.1.2.3 Reviewer's Comments and Evaluation

3.1.2.3.1 Sensitivity Analyses of 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders

Per request, the sponsor performed sensitivity analyses of 9/12 week abdominal pain and CSBM (APC) 3 + 1 responder.

The results from sensitivity analyses of f 9/12 week abdominal pain and CSBM (APC) 3 + 1 responder are given below.

9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders
Study LIN-MD-31

Analysis	PLA	LIN	Diff (RFX-PLA)	P-value
(LOCF)	22/395 (5.6%)	74/405 (18.3%)	12.7%	<0.0001
Completed Case	17/255 (6.7%)	41/246 (16.7%)	10.0%	0.0005
Observed Case	20/389 (5.1%)	49/393 (12.5%)	7.4%	0.0003
Worst Case 1	17/395 (4.3%)	41/405 (10.1%)	5.8%	0.0015
Worst Case 2	157/395 (39.7%)	41/405 (10.1%)	-29.6%	<0.00001
Worst Case 3	48/395 (12.2%)	49/405 (12.1%)	-0.1%	0.9931
Multiple Imputation	5.3%	17.7%	12.5%	<0.0001

Complied from Tables 14.4.1.1D-14.4.1.1I and 14.4.1.1K
P- values were obtained from the CMH tests controlling for geographic region.
The complete case analysis includes only those patients who complete at least 4 IVRS calls for each of the first 12 weeks of treatment.
The observed case analysis includes only those patients who complete at least 4 IVRS calls for at least one of the first 12 weeks of treatment.
For worst case analysis 1, patients must complete at least 4 IVARS calls for each of the first 12 weeks of treatment.
For worst case analysis 2, patients who do not complete at least 4 IVRS call for each of the first 12 weeks of treatment are handled as follows: patients randomized to Linaclotide are non-responders, while patients who are randomized to placebo are considered responders.
For worst case analysis 3, for those weeks where patients do not complete at least 4 IVRS calls, patients randomized to Linaclotide are non-responders, while patients who are randomized to placebo are considered responders.

The sponsor's worst case 1 analysis is one of "worst cast" analyses. It is more conservative than the sponsor's analysis.

As seen from the table above, for 9/12 week abdominal pain and CSBM (APC) 3 + 1 responder was shown by a significantly greater proportion of subjects taking linaclotide compared with subjects taking placebo in the worst case 1 analysis.

The sensitivity analyses using observed cases analysis showed similar results.

3.1.2.3.2 Subgroup Analysis of 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders for gender, age, race, BMI at baseline, and abdominal pain at baseline.

The summary of results of subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders is given below.

Subgroup Analyses of Proportion of 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders Study LIN-MD-31

Subgroup	Placebo	Linaclotidde	Diff (LIN-PLA)	95% CI
Gender				
Male	0/38 (0.0%)	4/38 (10.5%)	10.5%	(10.2%, 10.8%)
Female	20/357 (5.6%)	45/367 (12.2%)	6.7%	(6.5%, 6.8%)
Age				
<65	16/369 (4.3%)	47/386 (12.2%)	7.8%	(7.7%, 8.0%)
≥65	4/26 (15.4%)	2/19 (10.5%)	-4.9%	(-5.5%, -4.2%)
Race				
White	17/301 (5.6%)	41/314 (13.1%)	7.4%	(7.3%, 7.6%)
Black	2/75 (2.7%)	7/78 (9.0%)	6.3%	(6.1%, 6.5%)
Other	1/19 (5.3%)	1/13 (7.7%)	2.4%	(1.9%, 3.0%)
BMI at baseline				
< 30 kg/m^2	18/275 (6.5%)	25/271 (9.2%)	2.7%	(2.5%, 2.8%)
≥ 30 kg/m^2	2/120 (1.7%)	24/134 (17.9%)	16.2%	(16.0%, 16.5%)
Abdominal Pain at Baseline				
< 5	9/156 (5.8%)	16/152 (10.5%)	4.8%	(4.6%, 5.0%)
≥ 5 < 8	8/198 (4.0%)	31/214 (14.5%)	10.5%	(10.3%, 10.6%)
≥ 8	3/41 (7.3%)	2/39 (5.1%)	-2.2%	(-2.5%, -1.9%)

Compiled by this reviewer from Table 14.4.1.1J

As seen from the table above, 9 /12 week abdominal pain and CSBM(APC) 3 + 1 responder were reported by higher proportion of linaclotide subjects for gender, age <65, race, and BMI ≥30 kg/m^2 at baseline, abdominal pain at baseline (≥ 5 < 8).

3.1.2.3.3 Adequate Relief of Abdominal Pain and CSBM (APC) 3 + 1 Responder Rates by Week

As per request, the sponsor provided tabulation of number of subjects with adequate relief of abdominal pain and CSBM(APC) 3 + 1 by week through week 26 for ITT population (see below).

Weekly Abdominal Pain and CSBM (APC) 3 + 1 Responder Rate by Treatment Group Intention-to-Treat Population Study LIN-MD-31

	Study LIN-MD-31			
	PLA	**LIN**	**Diff** (LIN-PLA)	Chi-square p-value
Week 1	24/395 (6.1%)	70/405 (17.3%)	11.2%	<0.0001
Week 2	40/395 (10.1%)	82/405 (20.2%)	10.1%	<0.0001
Week 3	32/395 (8.1%)	102/405 (25.2%)	17.1%	<0.0001
Week 4	49/395 (12.4%)	114/405 (28.1%)	15.7%	<0.0001
Week 5	46/395 (11.6%)	101/405 (24.9%)	13.3%	<0.0001
Week 6	53/395 (13.4%)	110/405 (27.2%)	13.8%	<0.0001
Week 7	53/395 (13.4%)	96/405 (23.7%)	10.3%	<0.0001
Week 8	53/395 (13.4%)	98/405 (24.2%)	10.8%	<0.0001
Week 9	55/395 (13.9%)	92/405 (22.7%)	8.8%	0.0013
Week 10	46/395 (11.6%)	86/405 (21.2%)	9.6%	0.0002
Week 11	55/395 (13.9%)	88/405 (21.7%)	7.8%	0.0038
Week 12	43/395 (10.9%)	90/405 (22.2%)	11.3%	<0.0001

Compiled by this reviewer from Table 14.4.1.1C.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, greater proportions of subjects at almost every week during the course of the 12- week study in the linaclotide group compared with subjects in the placebo group was observed.

3.1.2.3.4 Adequate Relief of Abdominal Pain and CSBM (APC) + 1 Responder Rates by Week

As per request, the sponsor provided tabulation of number of subjects with adequate relief of abdominal pain and CSBM (APC) + 1 by week through week 26 for ITT population (see below).

Weekly Abdominal Pain and CSBM (APC) + 1 Responder Rate
by Treatment Group
Intention-to-Treat Population
Study LIN-MD-31

	Study LIN-MD-31			
	PLA	**LIN**	**Diff** (LIN-PLA)	Chi-square p-value
Week 1	39/395 (9.9%)	87/405 (21.5%)	11.6%	<0.0001
Week 2	69/395 (17.5%)	108/405 (26.7%)	9.2%	0.00016
Week 3	68/395 (17.2%)	134/405 (33.1%)	15.9%	<0.0001
Week 4	76/395 (19.2%)	143/405 (35.3%)	16.1%	<0.0001
Week 5	74/395 (18.7%)	140/405 (34.6%)	15.8%	<0.0001
Week 6	83/395 (21.0%)	147/405 (36.3%)	15.3%	<0.0001
Week 7	86/395 (21.8%)	130/405 (32.1%)	10.3%	0.0009
Week 8	87/395 (22.0%)	126/405 (31.1%)	9.1%	0.0033
Week 9	91/395 (23.0%)	130/405 (32.1%)	9.1%	0.0038
Week 10	75/395 (19.0%)	125/405 (30.9%)	11.9%	0.0002
Week 11	91/395 (23.0%)	129/405 (31.9%)	8.9%	0.0050
Week 12	81/395 (20.5%)	120/405 (29.6%)	9.1%	0.0026

Compiled by this reviewer from Table 14.4.1.4C.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, greater proportions of subjects at almost every week during the course of the 12- week study in the linaclotide group compared with subjects in the placebo group was observed.

3.1.2.3.5 Monthly Abdominal Pain and CSBM (APC) Responder Rate

This reviewer performed analyses of abdominal pain and CSBM(APC) by month. Subject with missing monthly responder at a specific month was assumed to be "failure" for that month.

The results from reviewer's analyses of abdominal pain and CSBM(APC) by month are given below.

Monthly Abdominal Pain and CSBM (APC) 3 + 1 Responder Rate
by Treatment Group
Intention-to-Treat Population
Study LIN-MD-31

	Study LIN-MD-31			
	PLA	**LIN**	**Diff** (LIN-PLA)	Chi-square p-value
Month 1	43/395 (10.9%)	106/405 (26.2%)	15.3%	<0.0001
Month 2	58/395 (14.7%)	112/405 (27.7%)	13.0%	<0.0001
Month 3	57/395 (14.4%)	102/405 (25.2%)	10.8%	0.0001

Compiled by this reviewer from Table 14.4.3.24B.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, for monthly abdominal pain and CSBM (APC) 3 + 1 responder, greater proportions of subjects at every month during the course of the 3-

month study in the linaclotide group compared with subjects in the placebo group was observed.

Monthly Abdominal Pain and CSBM (APC) + 1 Responder Rate by Treatment Group Intention-to-Treat Population Study LIN-MD-31

	Study LIN-MD-31			
	PLA	**LIN**	**Diff** (LIN-PLA)	Chi-square p-value
Month 1	79/395 (20.0%)	145/405 (35.8%)	15.8%	<0.0001
Month 2	95/395 (24.1%)	159/405 (39.3%)	15.2%	<0.0001
Month 3	97/395 (24.6%)	142/405 (35.1%)	10.5%	0.0010

Compiled by this reviewer from Table 14.4.3.27B.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, for monthly abdominal pain and CSBM (APC) + 1 responder, greater proportions of subjects at every month during the course of the 3-moth study in the linaclotide group compared with subjects in the placebo group was observed.

3.1.2.3.6 Sustained Efficacy – Monthly Abdominal Pain and CSBM (APC) Responder

3.1.2.3.6.1 Sustained Efficacy – At Least 2 of 3 Months

For sustained efficacy, the commonly used primary efficacy endpoint for IBS is "overall responder." A subject was considered an overall responder if the subject was a monthly responder for at least two out of any three months during 12-week study.

This reviewer performed analysis of overall responder for abdominal pain and CSBM (APC) 3 + 1 and abdominal pain and CSBM (APC) + 1. The results are given below.

Reviewer's Overall Responder Analysis by Treatment Group Intention-to-Treat Population Study LIN-MD-31

cEndpoint	**PLA** N=395	**LIN** n=405	**Diff** (LIN-PLA)	**p-value**
Abdominal Pain and CSBM (APC) 3 + 1 ≥ 2 Months	52 (13.2%)	107 (26.4%)	13.3%	<0.0001
Abdominal Pain and CSBM (APC) + 1 ≥ 2 Months	95 (24.1%)	153 (37.8%)	13.7%	<0.0001

Compiled by this reviewer from Table 14.4.3.24A and Table 14.-4.3.27A.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, for overall responder for monthly abdominal pain and CSBM (APC) 3 +1 responder and monthly abdominal pain and CSBM (APC) 3 +1 responder, respectively, greater proportions of subjects during the course of the 3-month study in the linaclotide group compared with subjects in the placebo group was observed.

3.1.2.3.6.2 Sustained Efficacy – All 3 Months

For sustained efficacy, a subject was considered an overall responder if the subject was a monthly responder for all 3 months during 12-week study. This definition is more stringent than previous definition for overall responder (at least 2 of 3 months).

This reviewer performed analysis of overall responder for abdominal pain and CSBM (APC) 3 +1 and abdominal pain and CSBM (APC) +1. The results are given below.

Reviewer's Overall Responder Analysis by Treatment Group
LIN-MD-31
Intention-to-Treat Population

Endpoint	PLA N=395	LIN n=405	Diff (LIN-PLA)	p-value
Abdominal Pain and CSBM (APC) 3 + 1 = 3 Months	22 (5.6%)	59 (14.6%)	9.0%	<0.0001
Abdominal Pain and CSBM (APC) + 1 = 3 Months	48 (12.2%)	92 (22.7%)	10.5%	<0.0001

Compiled by this reviewer
P-values were obtained by the Fisher's Exact test.

As seen from the table above, for overall responder for abdominal pain and CSBM (APC) 3 + 1 and abdominal pain and CSBM (APC) + 1, greater proportions of subjects in the linaclotide group compared with subjects in the placebo group was observed.

3.1.2.3.7 Reviewer's Comments on Sponsor's Controlling for Multiplicity for Primary and Secondary Efficacy Parameter

The sponsor used 5-step serial gatekeeping multiple comparison procedure to control type 1 family-wise error rate for testing the primary and secondary efficacy parameters.

The overall type I family-wise error rate for testing the primary and secondary efficacy parameters was controlled at the 0.05 significance level using the following 5-step serial gatekeeping multiple comparisons procedure (MCP). Following this MCP, progression to the next step only occurred if all individual null hypotheses within a step were rejected and the previous step(s) were all rejected at the step-specific overall significance level. If all null hypotheses within a step were not rejected, the statistical tests corresponding to all subsequent steps were considered not statistically significant. All hypothesis tests were two-sided.

1. The first step tested the 4 primary efficacy parameters using a fixed sequential testing method. The 4 primary efficacy parameters were each tested at the 0.05 significance level in the following fixed sequence:

    1. 9/12 Week APC 3+1 Responder
    2. 9/12 Week CSBM 3+1 Responder
    3. 9/12 Week Abdominal Pain Responder

4. 6/12 Week APC +1 Responder

If a null hypothesis was not rejected (i.e., p-value > 0.05), all subsequent statistical tests were not considered statistically significant.

2. The second step tested the following 4 secondary parameters:
   - Change from baseline in 12-week CSBM Frequency Rate
   - Change from baseline in 12-week SBM Frequency Rate
   - Change from baseline in 12-week Stool Consistency
   - Change from baseline in 12-week Severity of Straining

   These 4 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure (22) to control for multiple parameters within this step.

3. The third step tested the following 3 secondary parameters:
   - Change from baseline in 12-week Abdominal Pain
   - Change from baseline in 12-week Abdominal Discomfort
   - Change from baseline in 12-week Bloating

   These 3 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure (22) to control for multiple parameters within this step.

4. The fourth step tested the following 2 secondary parameters:
   - 6/12 Week CSBM +1 Responder
   - 6/12 Week Abdominal Pain Responder

   These 2 secondary parameters were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters within this step.

5. The fifth step tested the following single secondary parameter:
   - Change from baseline in 12-week Percent of Abdominal Pain-free Days

   This secondary parameter was tested using a type I error rate of 0.05.

This reviewer's comments on this gatekeeping procedure were:

> The sponsor's gatekeeping procedure was not appropriate. The Hochberg procedure is generally not recommended for sequential testing. It is not assumption free. Furthermore, it is known to provide overall α-control for independent and for certain types of positive correlated endpoints. But its properties for other types of dependent endpoints are not fully known. Various simulation experiments indicate that this method generally controls the overall Type 1 error rate for positive correlated endpoints but fails to do so for some negatively correlated endpoints.

The sponsor should use a Bonferroni based gatekeeping procedure to test all endpoints in the primary family and proceed to the secondary family of endpoints only if there has been statistical success in the primary family.

Furthermore, since p-values for most secondary endpoints were very small (<0.001), all secondary endpoints would pass any statistical procedure for controlling the type 1 error for multiplicity.

3.1.2.3.8 Reviewer's Comments on Results of Analyses of Secondary Efficacy Endpoints

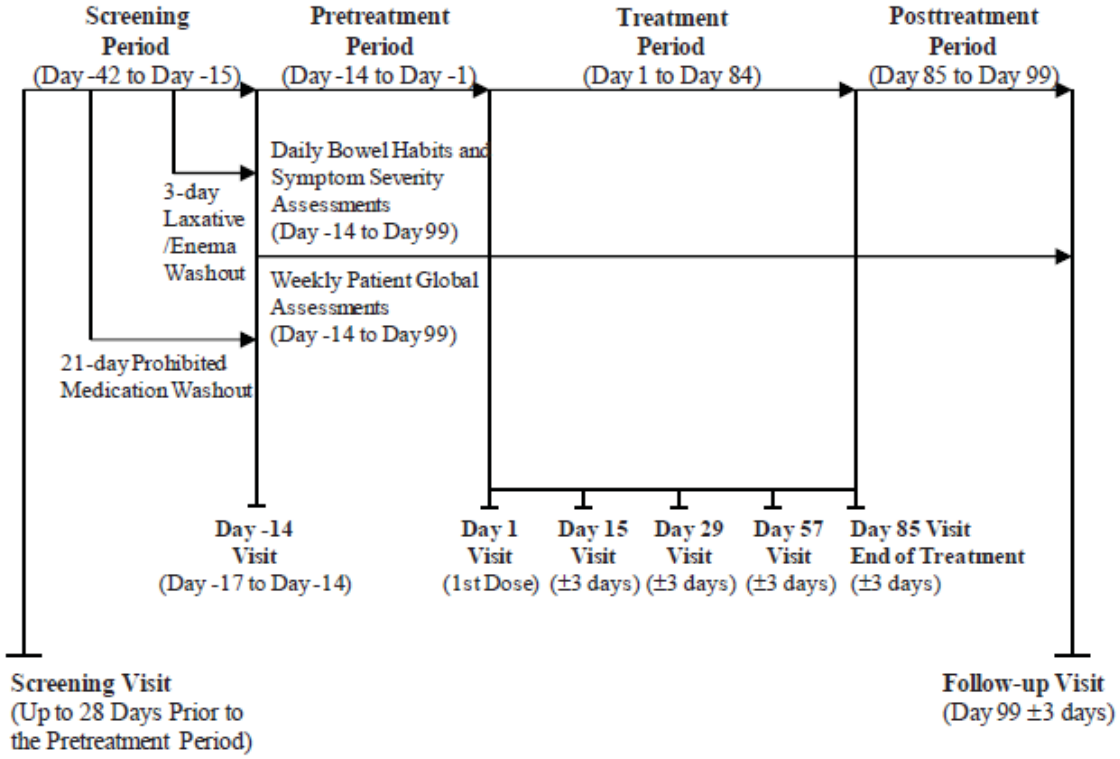
The sponsor's pre-specified analysis for the secondary endpoints was based on a modeling approach (ANCOVA) using all data for each week 1-12. The term "treatment overall" refers to an average treatment effect over the 12 weeks of the study. (b) (4)

3.1.3 Reviewer's Comments on Dose Selection

The sponsor had performed a phase 2 dosing ranging study, MCP-103-202-CSR-01. This study was a multicenter, randomized, double-blind, parallel-group, placebo-controlled, dose-range-finding oral dose study of 75, 150, 300, and 600 µg linaclotide administered to patients with IBS-C.

This study consisted of 4 distinct periods (Figure 1).

Figure 1. Illustration of Study Design – Study MCP-103-202-CSR-01



The primary efficacy endpoint was the change from baseline in the weekly normalized CSBM Rate during Weeks 1 through 12 of the Treatment Period.

For each week, the CSBM rate was normalized based on the number of CSBMs occurring in that week, adjusting for differences in the duration of the week and black-out periods (time not covered due to a missed IVRS call) versus 7x24 hours.

Endpoints based on CSBMs and SBMs were calculated as weekly rates. This facilitated the comparison of periods of different lengths, such as the 2-week Pretreatment Period and the 12-week Treatment Period. To compute weekly rates, the number of events was divided by the length of the period (in hours) and then multiplied by (7×24). For example, if a patient had 41 SBMs during the entire 12-week Treatment Period, and the Treatment Period lasted 84 days (84 days × 24 = 2,016 hours) then the patient's weekly SBM rate for the Treatment Period = (41 / 2,016) x (7×24) = 3.42 SBMs per week. This calculation provided the 'observed' rate. If a patient took a rescue medication, any BMs in the subsequent 24 hours would not be counted as SBMs in this calculation.

The calculation for the normalized rates took these 'black-out' intervals into account by subtracting the black-outs from the total length of the period.

The primary efficacy endpoint was analyzed using an Analysis of Covariance (ANCOVA) with a fixed effect term for treatment group and study center and baseline weekly normalized CSBM rate as a covariate. The baseline value was the overall

Pretreatment Period weekly normalized CSBM rate. No adjustments to the p-values for multiplicity were made.

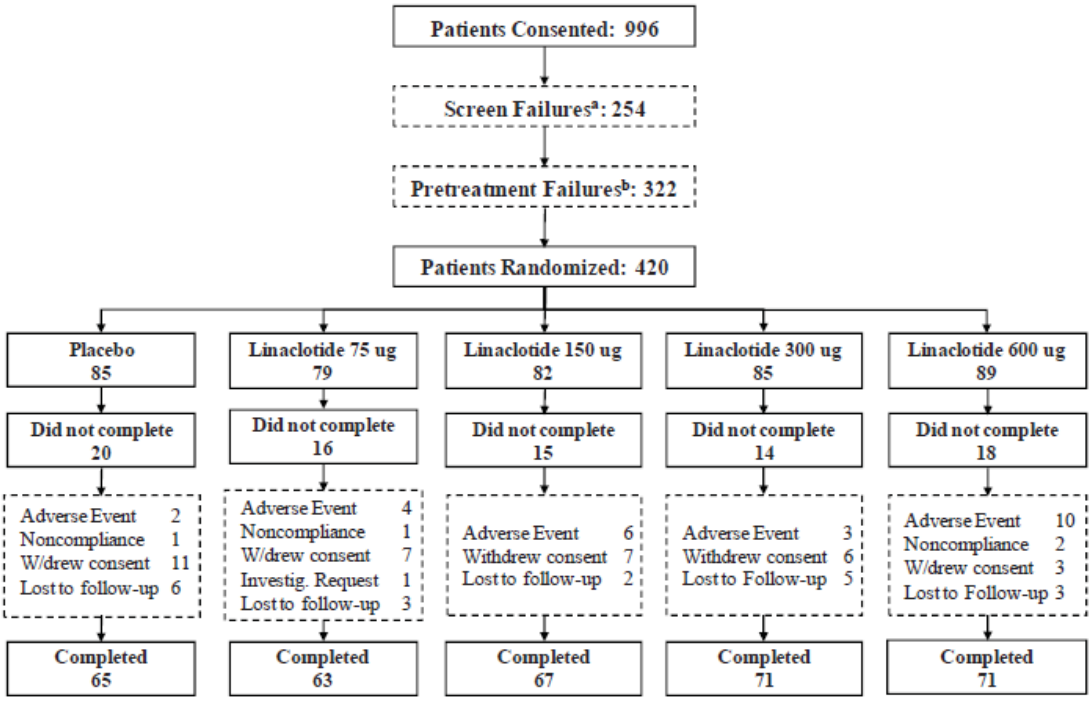
The primary efficacy analysis was based on the Evaluable Population.

For each of the 4 active linaclotide groups (75, 150, 300, and 600 µg), the null hypothesis was that there was no difference between placebo and the dose group in the change from baseline in the weekly normalized CSBM Rate. An observed cases (OC) approach to missing post-baseline data was applied: any missing data were not imputed.

A total of 420 patients were randomized (85 placebo, 79 linaclotide 75 µg, 82 linaclotide 150 µg, 85 linaclotide 300 µg, and 89 linaclotide 600 µg).

The patient disposition is given below.

Figure 2. Patient Disposition - Study MCP-103-202-CSR-01



Data Source: Section 14, Tables 14.1.1.1 and 14.1.1.2.

a Screen Failures were consented patients who did not qualify for inclusion into the study based on their screening evaluations. Patients who were re-screened and failed again were only counted once. Patients who initially failed at screening, were re-screened, and subsequently were either randomized or Pretreatment Period failures were not counted in this group.

b Pretreatment Failures were consented patients who entered the Pretreatment Period but were not randomized into the study.

Note: Patients who were re-screened and subsequently randomized were not counted in either 1 of the failure categories.

The primary analysis endpoint was the change in the weekly normalized CSBM Rate during Weeks 1 through 12 of the Treatment Period from the weekly normalized CSBM Rate obtained during the Pretreatment Period. This analysis was performed using the

Evaluable Population. A summary of results from primary endpoint was provided in Table 14 (below).

Table 14. Primary Endpoint: Mean Change from Pretreatment to Treatment Period in Weekly Normalized CSBM Frequency (Evaluable Population) Study MCP-103-202-CSR-01

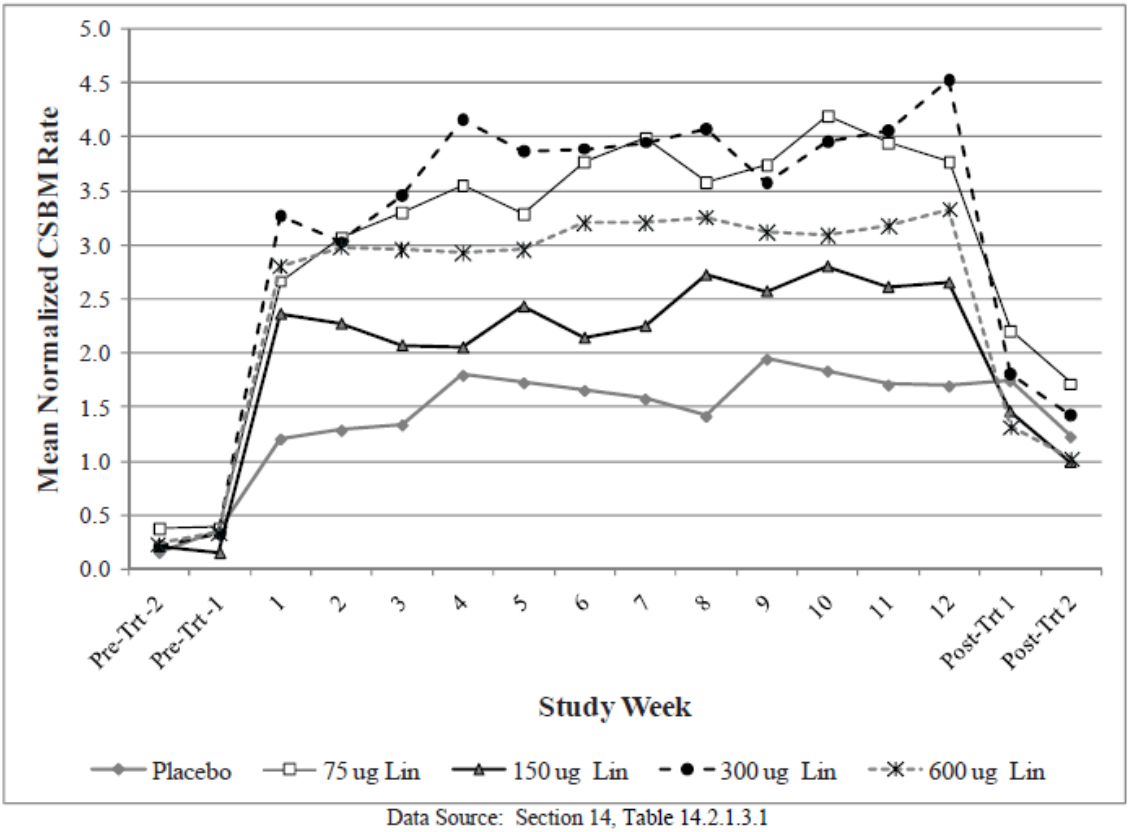
			Linaclotide				
		Placebo	75 ug	150 ug	300 ug	600 ug	All
Pretreatment Period	N	62	54	61	67	62	244
	Mean	0.25	0.38	0.18	0.26	0.28	0.27
	(SD)	(0.488)	(0.652)	(0.364)	(0.523)	(0.520)	(0.522)
	Median	0.00	0.00	0.00	0.00	0.00	0.00
	min,max	0.0, 2.4	0.0, 2.4	0.0, 1.4	0.0, 2.4	0.0, 2.5	0.0, 2.5
Treatment Period	N	62	54	61	67	62	244
	Mean	1.60	3.56	2.42	3.81	3.07	3.22
	(SD)	(1.849)	(3.438)	(2.621)	(3.590)	(2.924)	(3.195)
	Median	1.06	2.83	1.42	3.20	2.51	2.46
	min,max	0.0, 7.8	0.0, 19.1	0.0, 11.4	0.0, 19.5	0.0, 12.6	0.0, 19.5
LS Mean Change	N	62	54	61	67	62	
	Mean	1.32	3.05	2.26	3.51	2.74	
	(SE)	(0.376)	(0.409)	(0.384)	(0.364)	(0.391)	
	p-value[a]						0.0003
LS Mean Difference from Placebo	Mean		1.73	0.94	2.19	1.42	
	(SE)		(0.526)	(0.508)	(0.497)	(0.508)	
	p-value[b]		0.0011	0.0644	<.0001	0.0056	

Data Source: Section 14, Table 14.2.1.1.1
SE=standard error
a F test was based on the ANCOVA model to test for overall treatment effect.
b Pairwise p-values were based on a comparison of each linaclotide group versus the placebo group using ANCOVA.

The clinical report stated "For the 4 linaclotide groups in the Evaluable Population, increases from baseline in normalized weekly CSBM Rate were numerically superior to placebo and were independent of dose: 1.32 for placebo, 3.05 for 75 µg linaclotide, 2.26 for 150 µg linaclotide, 3.51 for 300 µg linaclotide, and 2.74 for 600 µg linaclotide (Table 14). The mean changes from the Pretreatment Period in weekly normalized CSBM Rate during the Treatment Period (calculated as the average of the 12 treatment weeks) were statistically significantly greater for the 75, 300, and 600 µg linaclotide groups compared with the placebo group."

Figure 4 presents weekly normalized CSBM Rate by dose group for the Pretreatment (baseline, Weeks -1 and -2), Treatment (Weeks 1 to 12), and Posttreatment (Weeks 13 and 14) Periods for the Evaluable Population.

Figure 4. Mean Weekly Normalized CSBM Rates, by Dose Group and Study Week (Evaluable Population)
Study MCP-103-202-CSR-01



Data Source: Section 14, Table 14.2.1.3.1

The clinical report stated "For all 4 linaclotide groups, statistically significant increases in CSBM Rate occurred during the first week of treatment versus the placebo group. For the 75 and 300 µg dose groups, statistically significant differences versus the placebo group in weekly CSBM Rate were maintained throughout the entire 12 weeks of the Treatment Period. For the 150 and 600 µg dose groups the improvements were numerically greater than the placebo group throughout the entire 12 weeks of the Treatment Period. Sustained CSBM Rate increases of a lesser magnitude were also observed for the placebo group throughout the Treatment Period. Mean CSBM Rate for all dose groups remained slightly elevated during the Posttreatment Period when compared with the Pretreatment Period."

This reviewer's comments on results of study are as follows:

> For the primary endpoint, all doses except linaclotide 150 µg achieved statistical significance at nominal significance level of 0.05. The p-value for linaclotide 150 µg was just barely larger than 0.05 (0.0644).
>
> As seen from the Figure 4, there was no separation between linaclotide 75 µg and linaclotide 300 µg for mean weekly normalized CSBM rates by week.

The data revealed that linaclotide 300 μg might not be the lowest effective dose.

3.2 Evaluation of Safety

3.2.1 Study MCP-103-302

Serious adverse events (SAEs) were experienced by 4 linaclotide patients (rotator cuff syndrome, appendicitis, cystopexy, and stage IV nodular sclerosing-type Hodgkin's disease in 1 patient each) and by 7 placebo patients (transient ischemic attack, uterine leiomyoma, angioedema, vertigo, lower abdominal pain, and stage IV rectal cancer in 1 patient each; bronchitis and viral pneumonia in 1 patient). No patient experienced an SAE that was considered by the Investigator to be related to study drug.

During the Treatment Period, 65.4% of linaclotide patients experienced at least 1 treatment-emergent AE (TEAE), compared with 56.6% of placebo patients. The only TEAEs experienced by at least 3% of linaclotide patients, and at an incidence greater than that in placebo patients, were diarrhea (19.7% vs. 2.5%), abdominal pain (4.5% vs. 4.0%), flatulence (3.7% vs. 2.2%), viral gastroenteritis (3.7% vs. 2.2%), and headache (3.2% vs. 2.7%). Of the 79 linaclotide patients who had TEAEs of diarrhea during the treatment period, 50 patients (63.3%) experienced the onset of diarrhea during the first 2 weeks of treatment.

The majority of the TEAEs in both treatment groups were reported as mild or moderate in severity. A total of 31 patients (7.7%) in the linaclotide group and 19 patients (4.7%) in the placebo group experienced at least 1 severe TEAE. TEAEs that were reported as severe in at least 2 linaclotide patients were diarrhea (8 patients; 2.0%), abdominal pain (3 patients; 0.7%), and abdominal distension (2 patients; 0.5%). TEAEs that resulted in the discontinuation of at least 1% of linaclotide patients were diarrhea (4.5% vs. 0.2% for placebo patients) and abdominal pain (1.2% vs. no placebo patients).

Per this reviewer's request, the sponsor performed analysis of number of patients with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea.

The results are given below.

Number of Patients with at Least One AE, at Least One TRAE. Withdrawn due to AE, at Least One Episode of Diarrhea, and Discontinued due to TRAE of Diarrhea
MCP-103-302

Parameter	Placebo (N=403) n (%)	LIN 290 ug (N=402) n (%)	Odds Ratio [Exact 95% CI for Odds Ratio (Lin 290 : Placebo)]
Number of Patients with at Least One AE	228 (56.6)	263 (65.4)	1.45 (1.08 , 1.95)
p-value		0.0114	
Number of Patients with at Least One TRAE	58 (14.4)	120 (29.9)	2.53 (1.76 , 3.66)
p-value		<.0001	
Number of Patients Withdrawn due to AE	8 (2.0)	40 (10.0)	5.46 (2.47 , 13.65)
p-value		<.0001	
Number of Patients with at Least One Episode of Diarrhea	10 (2.5)	81 (20.1)	9.92 (5.01 , 21.77)
p-value		<.0001	
Number of Patients Discontinued due to TRAE of Diarrhea	1 (0.2)	18 (4.5)	18.84 (2.94 ,787.02)
p-value		<.0001	

Copied from Table 9.4

As seen from the table above, for number of patients with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea, greater proportions of subjects in the linaclotide group compared with subjects in the placebo group was observed.

3.2.2 Study LIN-MD-31

With the exception of patient discontinuations as a result of an AE, the incidences of TEAEs and SAEs were similar between treatment groups.

There were 3 TEAEs (diarrhea, abdominal pain, and flatulence) which had a meaningful difference in incidence between the treatment groups.

Diarrhea was the most frequently reported TEAE among patients treated with linaclotide; 79 (19.5%) patients in the linaclotide group experienced at least 1 episode of diarrhea vs. only 14 (3.5%) patients treated with placebo. A total of 8 of the 79 patients treated with linaclotide who experienced TEAEs of diarrhea had events that were reported as severe. A total of 23 (5.7%) patients treated with linaclotide discontinued from the study because of a TEAE of diarrhea versus only 1 (0.3%) placebo patient.

Per this reviewer's request, the sponsor performed analysis of number of patients with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea.

The results are given below.

Number of Patients with at Least One AE, at Least One TRAE. Withdrawn due to AE, at Least One Episode of Diarrhea, and Discontinued due to TRAE of Diarrhea Study LIN-MD-31

Parameter	Placebo (N=396) n (%)	LIN 290 ug (N=406) n (%)	Odds Ratio [Exact 95% CI for Odds Ratio (Lin 290 : Placebo)]
Number of Patients with at Least One AE	210 (53.0)	228 (56.2)	1.13 (0.85 , 1.51)
p-value		0.3949	
Number of Patients with at Least One TRAE	57 (14.4)	123 (30.3)	2.58 (1.80 , 3.74)
p-value		<.0001	
Number of Patients Withdrawn due to AE	11 (2.8)	31 (7.6)	2.89 (1.39 , 6.47)
p-value		0.0023	
Number of Patients with at Least One Episode of Diarrhea	14 (3.5)	79 (19.5)	6.59 (3.61 , 12.82)
p-value		<.0001	
Number of Patients Discontinued due to TRAE of Diarrhea	1 (0.3)	23 (5.7)	23.72 (3.80 ,979.55)
p-value		<.0001	

Copied from Table 9.4

As seen from table above, for number of patients with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea, greater proportions of subjects in the linaclotide group compared with subjects in the placebo group was observed.

4. FINDINGS IN SPECIAL/SUBGROUP POPULATION

4.1 Gender, Race and Age

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 9/12 week abdominal pain and CSBM(APC) 3 +1 responders for gender, age, and race, BMI at baseline, and abdominal pain at baseline.

4.1.1 Study MCP-103-302

The summary of results of subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders for Study MCP-103-302 is given below.

Subgroup Analyses of Proportion of 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders Study MCP-103-302

Subgroup	Placebo	Linaclotidde	Diff (LIN-PLA)	95% CI
Gender				
Male	3/51 (5.9%)	5/33 (15.2%)	9.3%	(8.8%, 9.7%)
Female	9/352 (2.6%)	46/368 (12.5%)	9.9%	(9.8%, 10.1%)
Age				
<65	12/386 (3.1%)	47/378 (12.4%)	9.3%	(9.2%, 9.4%)
≥65	0/17 (0.0%)	4/23 (17.4%)	17.4%	(16.9%, 17.9%)
Race				
White	9/311 (2.9%)	43/316 (13.6%)	10.7%	(10.6%, 10.8%)
Black	2/78 (2.6%)	7/70 (10.0%)	7.4%%	(7.2%, 7.7%)
Other	1/14 (7.1%)	1/15 (6.7%)	-0.4%	(-1.1%, 0.1%)

Compiled by this reviewer from Table 14.4.1.1J

As seen from the table above, 9/12 week abdominal pain and CSBM (APC) 3 + 1 responder were reported by higher proportion of linaclotide subjects for gender, age and race.

4.1.2 Study LIN-MD-31

The summary of results of subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders for Study LIN-MD-31 is given below.

Subgroup Analyses of Proportion of 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders Study LIN-MD-31

Subgroup	Placebo	Linaclotidde	Diff (LIN-PLA)	95% CI
Gender				
Male	0/38 (0.0%)	4/38 (10.5%)	10.5%	(10.2%, 10.8%)
Female	20/357 (5.6%)	45/367 (12.2%)	6.7%	(6.5%, 6.8%)
Age				
<65	16/369 (4.3%)	47/386 (12.2%)	7.8%	(7.7%, 8.0%)
≥65	4/26 (15.4%)	2/19 (10.5%)	-4.9%	(-5.5%, -4.2%)
Race				
White	17/301 (5.6%)	41/314 (13.1%)	7.4%	(7.3%, 7.6%)
Black	2/75 (2.7%)	7/78 (9.0%)	6.3%	(6.1%, 6.5%)
Other	1/19 (5.3%)	1/13 (7.7%)	2.4%	(1.9%, 3.0%)

Compiled by this reviewer from Table 14.4.1.1J

As seen from the table above, 9/12 week abdominal pain and CSBM (APC) 3 + 1 responder were reported by higher proportion of linaclotide subjects for gender and race.

4.2 Other Special/Subgroup Population

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders for BMI at baseline and abdominal pain at baseline.

4.2.1 Study MCP-103-302

The summary of results of subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders for Study MCP-103-302 is given below.

Subgroup Analyses of Proportion of 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responders Study MCP-103-302

Subgroup	Placebo	Linaclotidde	Diff (LIN-PLA)	95% CI
BMI at baseline				
< 30 kg/m^2	7/285 (2.5%)	35/280 (12.5%)	10.0%	(9.9%, 10.2%)
≥ 30 kg/m^2	5/118 (4.2%)	16/121 (13.2%)	9.0%	(8.8%, 9.2%)
Abdominal Pain at Baseline				
< 5	4/176 (2.3%)	21/165 (12.7%)	10.4%	(10.3%, 10.6%)
$\geq 5 < 8$	7/185 (3.8%)	27/189 (14.3%)	10.5%	(10.3%, 10.7%)
≥ 8	1/42 (2.4%)	3/47 (6.4%)	4.0%	(-3.7%, 4.3%)

Compiled by this reviewer from Table 14.4.1.1J

As seen from the table above, 9/12 week abdominal pain and CSBM(APC) 3 + 1 responder were reported by higher proportion of linaclotide subjects for BMI at baseline and abdominal pain at baseline (<5 and $\geq 5 < 8$).

4.2.1 Study LIN-MD-31

The summary of results of subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders for Study LIN-MD-31 is given below.

Subgroup Analyses of Proportion of 9/12 Week Abdominal Pain And CSBM (APC) 3+1 Responders Study LIN-MD-31

Subgroup	Placebo	Linaclotidde	Diff (LIN-PLA)	95% CI
BMI at baseline				
< 30 kg/m^2	18/275 (6.5%)	25/271 (9.2%)	2.7%	(2.5%, 2.8%)
≥ 30 kg/m^2	2/120 (1.7%)	24/134 (17.9%)	16.2%	(16.0%, 16.5%)
Abdominal Pain at Baseline				
< 5	9/156 (5.8%)	16/152 (10.5%)	4.8%	(4.6%, 5.0%)
$\geq 5 < 8$	8/198 (4.0%)	31/214 (14.5%)	10.5%	(10.3%, 10.6%)
≥ 8	3/41 (7.3%)	2/39 (5.1%)	-2.2%	(-2.5%, -1.9%)

Compiled by this reviewer from Table 14.4.1.1J

As seen from the table above, 9/12 week abdominal pain and CSBM(APC) 3 + 1 responder were reported by higher proportion of linaclotide subjects for BMI at baseline (≥ 30 kg/m^2) and abdominal pain at baseline (<5 and $\geq 5<8$).

5. SUMMARY AND CONCLUSIONS

5.1 Statistical Issues and Collective Evidence

Study MCP-103-302 showed that linaclotide was statistically significantly better than placebo in terms of the primary efficacy endpoint, 9/12 week APC 3+1 Responder. The treatment difference was 9.7%. It was also statistically better than placebo in terms of other three primary efficacy endpoints: 9/12 week CSBM 3+1 Responder, 9/12 week Abdominal Pain Responder, and 6/12 week APC +1 Responder. The treatment differences ranged from 13% to 20%. The superiority was also shown for some secondary efficacy endpoints: change from baseline in 12-week CSBM frequency rate, change from baseline in 12-week SBM frequency rate, change from baseline in 12-week stool consistency, CSBM frequency rate, and change from baseline in 12-week percent of abdominal pain-free days.

The efficacy results from Study MCP-103-302 were replicated in Study LIN-MD-31 for primary efficacy endpoint: 9/12 week APC 3+1 Responder. However, the treatment difference was modest at 7.0%.

It was found the sponsor failed to perform subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders by gender, age, and race.

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 9/12 week abdominal pain and CSBM (APC) 3 +1 responders for gender, age, race, BMI at baseline, and abdominal pain at baseline.

Results from subgroup analyses show that treatment effect was consistent between studies for gender, age (<65), race, BMI at baseline (≥ 30 kg/m^2) and abdominal pain (<5 and ≥ 5 < 8).

It was found that the sponsor failed to perform number of subjects with adequate relieve pain and CSBM (APC) 3 +1 by week and by month. A subject was considered to be monthly responder if subject was weekly responder for at least 2 of 4 weeks at that month.

As per request, the sponsor provided analysis of number of subjects with adequate relieve pain and CSBM (APC) 3 +1 by week and by month. Greater proportions of patients at almost every week and every month during the 26-week study in the linaclotide group as compared with patients in the placebo was observed in Study MCP-103-302. Similar results were observed during the 12-weekly study in Study LIN-MD-31.

This review performed post-hoc analyses for sustained efficacy for both study. A subject was considered sustained responder if subject was monthly responder for all 3 months. Greater proportions of patients in the linaclotide group as compared with patients in the placebo was observed in both studies for both endpoints of abdominal pain CSBM (APC) 3 + 1 and abdominal pain CSBM (APC) + 1. The treatment differences ranged from 9.0% to 12.8% for abdominal pain CSBM (APC) 3 + 1 and from 10.5% to 17.0% for abdominal pain CSBM (APC) 3 + 1.

For safety, for number of patients with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea, greater proportions of subjects in the linaclotide group compared with subjects in the placebo group was observed in both studies (MCP-103-302 and LIN-MD-31).

5.2 Conclusions and Recommendations

In conclusion, both studies (MCP-103-302 and LIN-MD-31) showed that linaclotide was superior to the placebo for protocol-specified endpoint, 9/12 week APC 3+1 responder. The treatment difference was 9.7 and 7.0 for studies MCP-103-302 and LIN-MD-31, respectively.

Both studies also showed that linaclotide (266 μg) was statistically significantly better than placebo in terms of other three primary efficacy endpoints: 9/12 week CSBM 3+1 responder, 9/12 week abdominal pain responder, and 6/12 week APC +1 responder. The treatment differences ranged from 13% to 20%. Superiority was also shown for some secondary efficacy endpoints: change from baseline in 12-week CSBM frequency rate, change from baseline in 12-week SBM frequency rate, change from baseline in 12-week stool consistency, CSBM frequency rate, and change from baseline in 12-week percent of abdominal pain-free days.

The efficacy results from Study MCP-103-302 were replicated in Study LIN-MD-31 for primary efficacy endpoint: 9/12 week APC 3+1 responder. However, the treatment difference was modest with 7.0%.

As per request, the sponsor provided analysis of number of subjects with adequate relieve pain and CSBM (APC)3 +1 by week and by month. Greater proportions of patients at almost every week and every month during the 26-week study in the linaclotide group as compared with patients in the placebo were observed in Study MCP-103-302. Similar results were observed during the 12-weekly study in Study LIN-MD-31.

In conclusion, both studies (MCP-103-302 and LIN-MD-31) showed that linaclotide was superior to the placebo for protocol-specified endpoint, 9/12 week APC 3+1 Responder. The treatment differences were 9.7% and 7.0% for studies MCP-103-302 and LIN-MD-31, respectively.

For safety, for number of patients with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea, greater proportions of subjects in the linaclotide group compared with subjects in the placebo group was observed in both studies (MCP-103-302 and LIN-MD-31).

Regarding safety concerns, the lower dose of linaclotide (133 μg) should have been included in these studies, since the lower dose was included for studies for chronic idiopathic constipation (CIC) and results from CIC studies showed no treatment difference between low dose and high dose in one of two pivotal phase III studies. It is suggested that the lower dose should be considered to be studied in the future.

6. APPENDIX

Table 1 Demographic and Baseline Characteristics – Safety Population Study MCP-103-302

Demographic Characteristic	Placebo (N=403)	Linaclotide (N=401)	Total (N=804)	p-value
Age, years				
Mean (SD)	44.0 (13.4)	44.6 (13.1)	44.3 (13.3)	0.4695
Median (Min, Max)	44.0 (18, 87)	45.0 (19, 82)	44.0 (18, 87)	
Age, n (%)				
18 to < 40 years	153 (38.0)	142 (35.4)	295 (36.7)	
40 to < 65 years	233 (57.8)	236 (58.9)	469 (58.3)	0.5174
≥ 65 years	17 (4.2)	23 (5.7)	40 (5.0)	
Gender, n (%)				
Female	352 (87.3)	368 (91.8)	720 (89.6)	0.0379
Male	51 (12.7)	33 (8.2)	84 (10.4)	
Race, n (%)				
Asian	6 (1.5)	2 (0.5)	8 (1.0)	
Black/African American	78 (19.4)	70 (17.5)	148 (18.4)	0.5619
Caucasian	311 (77.2)	316 (78.8)	627 (78.0)	
Other	8 (2.0)	13 (3.2)	21 (2.6)	
Ethnicity, n (%)				
Hispanic/Latino	38 (9.4)	43 (10.7)	81 (10.1)	0.5390
Not Hispanic/Latino	365 (90.6)	358 (89.3)	723 (89.9)	
Height, cm				
Mean (SD)	165.8 (7.8)	164.7 (7.9)	165.2 (7.9)	0.0343
Median (Min, Max)	165.1 (139.7, 193.0)	165.1 (134.6, 188.0)	165.1 (134.6, 193.0)	
Weight, kg				
Mean (SD)	76.4 (18.4)	75.5 (18.1)	75.9 (18.3)	0.4924
Median (Min, Max)	73.0 (43.9, 142.5)	72.1 (43.6, 173.6)	72.6 (43.6, 173.6)	
BMI, kg/m^2				
Mean (SD)	27.7 (6.2)	27.8 (5.9)	27.7 (6.1)	0.9348
Median (Min, Max)	26.5 (16.4, 54.2)	26.6 (17.7, 51.0)	26.6 (16.4, 54.2)	

Data Source: Section 14, Table 14.2.2

Age was calculated up to the informed consent date.

p-values for continuous variables (e.g., age, weight, height, BMI) were from an ANOVA with treatment group and region as factors; p-values for categorical variables (e.g., sex, ethnicity, and race) were from a CMH test controlling for geographic region.

SD = Standard Deviation, Min = Minimum, Max = Maximum, BMI = Body mass index, defined as weight in kg divided by height in m^2.

Table 2 Efficacy Variables at Baseline – ITT Population Study MCP-103-302

Efficacy Parameter	Statistic	Placebo (N=403)	Linaclotide (N=401)	Total (N=804)	p-value
Weekly CSBM Rate	n	403	401	804	0.2080
	Mean (SD)	0.2 (0.4)	0.2 (0.4)	0.2 (0.4)	
	Median	0.0	0.0	0.0	
	Min, Max	0.0, 2.9	0.0, 2.4	0.0, 2.9	
Weekly SBM Rate	n	403	401	804	0.9748
	Mean (SD)	1.7 (1.4)	1.7 (1.4)	1.7 (1.4)	
	Median	1.5	1.5	1.5	
	Min, Max	0.0, 5.4	0.0, 5.8	0.0, 5.8	
Stool Consistency (BSFS)	n	344	342	686	0.2499
	Mean (SD)	2.3 (1.0)	2.4 (1.1)	2.3 (1.0)	
	Median	2.0	2.0	2.0	
	Min, Max	1.0, 6.0	1.0, 6.0	1.0, 6.0	
Straining	n	344	342	686	0.6346
	Mean (SD)	3.5 (0.8)	3.6 (0.8)	3.6 (0.8)	
	Median	3.6	3.6	3.6	
	Min, Max	1.0, 5.0	1.0, 5.0	1.0, 5.0	
Abdominal Pain	n	403	401	804	0.4525
	Mean (SD)	5.5 (1.7)	5.6 (1.7)	5.6 (1.7)	
	Median	5.3	5.4	5.4	
	Min, Max	2.9, 10.0	2.9, 10.0	2.9, 10.0	
Percent of Abdominal Pain Free Days	n	403	401	804	0.9702
	Mean (SD)	2.1 (6.3)	2.1 (7.0)	2.1 (6.7)	
	Median	0.0	0.0	0.0	
	Min, Max	0.0, 57.1	0.0, 53.8	0.0, 57.1	
Abdominal Discomfort	n	403	401	804	0.2282
	Mean (SD)	6.0 (1.7)	6.1 (1.7)	6.1 (1.7)	
	Median	5.8	6.1	5.9	
	Min, Max	2.1, 10.0	2.5, 10.0	2.1, 10.0	
Bloating	n	403	401	804	0.2304
	Mean (SD)	6.5 (1.8)	6.6 (1.9)	6.6 (1.8)	
	Median	6.5	6.6	6.6	
	Min, Max	1.6, 10.0	0.0, 10.0	0.0, 10.0	

Data Source: Section 14, Table 14.2.4

Baseline efficacy values are derived from the IVRS data collected daily in the Pretreatment Period, specifically the period of time from 14 days before randomization up to the time of randomization.

SD = Standard Deviation, Min = Minimum, and Max = Maximum.

Table 3 Demographic and Baseline Characteristics – Safety Population
Study LIN-MD-31

Characteristic	*Placebo (N = 396)*	*Linaclotide (N = 406)*	*Total (N = 802)*	*P-value*
Age, years				
Mean ± SD	43.7 ± 12.9	43.3 ± 12.7	43.5 ± 12.8	0.6528
≥ 65 years, n (%)	26 (6.6)	19 (4.7)	45 (5.6)	0.3832
Range	18, 84	19, 81	18, 84	
Sex, n (%)				
Male	38 (9.6)	38 (9.4)	76 (9.5)	0.9295
Female	358 (90.4)	368 (90.6)	726 (90.5)	
Race, n (%)				
Caucasian	302 (76.3)	315 (77.6)	617 (76.9)	0.6391
Non-Caucasian	94 (23.7)	91 (22.4)	185 (23.1)	
Ethnicity, n (%)				
Hispanic	57 (14.4)	56 (13.8)	113 (14.1)	0.8354
Non-Hispanic	339 (85.6)	350 (86.2)	689 (85.9)	
Weight, kg				
Mean ± SD	74.6 ± 18.3	77.2 ± 18.8	75.9 ± 18.6	0.0375
Height, cm				
Mean ± SD	164.3 ± 8.3	165.2 ± 8.3	164.7 ± 8.3	0.1186
BMI. kg/m^2				
Mean ± SD	27.6 ± 6.2	28.3 ± 6.4	27.9 ± 6.3	0.1172

BMI = body mass index.

P-values for continuous variables (eg, age, weight, height, BMI) were from an ANOVA with treatment group and geographic region as factors; p-values for categorical variables (eg, sex, ethnicity, and race) were from a CMH test controlling for geographic region.

Source: Table 14.2.1.

Table 4 Efficacy Variables at Baseline – ITT Population Study LIN-MD-31

Parameter	*Placebo (N = 395)*	*Linaclotide (N = 405)*	*P-value*
	Mean ± SD	*Mean ± SD*	
CSBM rate per week	0.24 ± 0.50	0.20 ± 0.46	0.3149
SBM rate per week	1.90 ± 1.40	1.94 ± 1.38	0.6937
BSFS	2.41 ± 1.03	2.26 ± 1.00	0.0463
Straining score	3.43 ± 0.81	3.57 ± 0.76	0.0196
Abdominal pain	5.63 ± 1.71	5.66 ± 1.65	0.8553
Abdominal pain-free days	1.69 ± 6.00	2.06 ± 6.48	0.3971
Abdominal discomfort score	6.04 ± 1.67	6.17 ± 1.60	0.2734
Bloating score	6.50 ± 1.89	6.71 ± 1.77	0.0996

Baseline efficacy values were derived from the IVRS daily diary data collected in the pretreatment period, specifically the period from 14 days before randomization up to the time of randomization.

BSFS = Bristol Stool Form Scale; CSBM = complete spontaneous bowel movement; ITT = intent-to-treat; SBM = spontaneous bowel movement.

Source: Table 14.2.4.

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/s/

MILTON C FAN
08/16/2012

MICHAEL E WELCH
08/16/2012
Concur with review.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/BLA:	202-811
Drug Name:	Linzess (Linaclotide) 266 µg
Indication(s):	Treatment of chronic idiopathic constipation
Applicant:	Forest Laboratories, Inc.
Date(s):	Received August 9, 2011 PDUFA: September 9, 2012
Review Priority:	Standard
Biometrics Division:	Division of Biometrics III
Statistical Reviewer:	Milton C. Fan, Ph.D. , DB III
Concurring Reviewers:	Mike Welch, Ph.D. , Deputy Dir., DB III
Medical Division:	Gastroenterology and Inborn Errors Products (DGIEP)
Clinical Team:	Eric Wynn, M.D.,Robert Fiorentino, M.D. (TL) (DGIEP)
Project Manager:	Brian Strongin (DGIEP)

Keywords: clinical studies, NDA review, placebo controlled

Table of Contents

1. EXECUTIVE SUMMARY

1.1 Conclusions and Recommendations

The sponsor has submitted two pivotal studies (MCP-103-303 and LIN-MD-01) to support the indication for treatment of chronic idiopathic constipation (CIC). A separate statistical review addresses the IBS-C indication.

Study LIN-MD-01 showed that both linaclotide dose groups (133 μg and 266 μg) were statistically significantly better than placebo in terms of the primary efficacy endpoint, overall CSBM responder (See Section 1.2.1. for definition of overall responder.) The treatment differences were 9.9% and 14.7% for the linaclotide 133 μg and 266 μg, respectively.

Superiority was also shown for some secondary efficacy endpoints: change from baseline in 12-week CSBM frequency rate, change from baseline in 12-week SBM frequency rate, and change from baseline in 12-week stool consistency.

The treatment effects for the CSBM and SBM frequency rates were numerically greater for the higher dose group, however, a clinically meaningful dose response difference might not be evident.

The efficacy results from Study LIN-MD-01 were replicated in Study MCP-103-303 for the primary efficacy endpoint: 12-week CSBM overall responder rate. The treatment differences were 16.9% and 15.6% for the linaclotide 133 μg and 266 μg treatment groups, respectively.

This reviewer performed post-hoc analyses using a more stringent definition of responder, requiring subjects to be monthly responders for all 3 months. A subject was considered to be a monthly responder if the subject was weekly responders for at least 3 weeks of 4 weeks in the month.

For this more stringent definition, both linaclotide doses were superior to the placebo in both studies. Treatment differences were 7.5% and 12.8%, for linaclotide 133 μg and 266 μg dose groups, respectively in Study LIN-MD-01. Similar treatment differences were observed in Study MCP-103-303 (10.0% and 9.1%).

In conclusion, both studies (MCP-103-303 and LIN-MD-01) showed that both linaclotide doses (133 μg and 266 μg) were superior to placebo for the protocol-specified primary efficacy endpoint.

Regarding safety, greater proportions of subjects with adverse events were observed in the linaclotide groups compared with the placebo group for both studies. Specifically, more linaclotide subjects had at least one treatment related AE (TRAE), withdrew due to AE, had at least one episode of diarrhea, or discontinued due to a TRAE of diarrhea.

1.2. Brief Overview of Clinical Studies

1.2.1 Study LIN-MD-01

This is a phase 3, randomized, double-blind, placebo-controlled, parallel-group trial of Linaclotide administered orally for 12- weeks trial comparing 2 doses of linaclotide with placebo in patients with chronic idiopathic constipation (modified Rome II criteria). The trial was conducted in the U.S. (95 sites) and Canada (8 sites).

The objective of this trial was to determine the efficacy and safety of linaclotide administered to patients with chronic idiopathic constipation.

The trial consisted of up to 21 days of screening, 14 to 21 days of pretreatment, and 12 weeks of double-blind treatment. At the end of the pretreatment period, patients meeting the entry criteria were randomized to 1 of 3 double-blind treatment groups: 133 μg linaclotide, 266 μg linaclotide, or placebo (1:1:1). During this pretreatment period subjects provided qualified bowel habit and rescue medicine information.

An interactive voice response system (IVRS) was used for the primary efficacy assessment, and in particular to classify bowel movements (BMs) as spontaneous (SBMs) or complete (CSBMs).

The primary efficacy parameter was the 12-week CSBM overall responder rate. A 12-week CSBM overall responder was defined as a patient who was a CSBM weekly responder for at least 9 of the 12 weeks of the treatment period. A CSBM weekly responder was a subject who had a CSBM weekly frequency rate that was 3 or greater and increased by 1 or more from baseline.

1.2.2 Study MCP-103-303

The study design for this study was similar to that for Study LIN-MD-01 with exceptions listed below. The trial was conducted in the U.S in 110 sites.

This study was a phase 3, randomized, double-blind, placebo-controlled, parallel-group trial of 133 μg/day and 266 μg/day linaclotide administered orally for 12 Weeks followed by a 4-Week randomized withdrawal period.

The trial consisted of the following periods: up to 21 days of screening (screening period), 14 to 21 days of pretreatment (pretreatment period), 12 weeks of double-blind treatment (treatment period), and a 4-week double-blind randomized withdrawal (RW) period.

1.3 Statistical Issues and Findings

Study LIN-MD-01 showed that both linaclotide dose groups (133 μg and 266 μg) were statistically significantly better than placebo in terms of the primary efficacy endpoint, 12-week CSBM overall responder. The treatment differences were 9.9%

and 14.7% for linaclotide 133 µg and 266 µg, respectively. Superiority was also shown for some secondary efficacy endpoints: change from baseline in 12-week CSBM frequency rate, change from baseline in 12-week SBM frequency rate, and change from baseline in 12-week stool consistency.

For the changes from baseline in CSBMs/week and SBMs/week, the treatment effects were slightly numerically greater for subjects in the 266 µg dose group than for those in the 133 µg dose group. For CSBM, the linaclotide treatment effects were 1.4 and 2.0 for the 133 µg and 266 µg dose groups, respectively, and the SBM effects were 2.3 and 2.6. The within-dose differences in these group effects (0.6 and 0.3) might not be considered clinical meaningful.

The efficacy results from Study LIN-MD-01 were replicated in Study MCP-103-303 for primary efficacy endpoint: 12-week CSBM overall responder. However, the treatment differences were 16.9% and 15.6% for linaclotide 133 µg and 266 µg, respectively.

This reviewer performed analyses of CSBM monthly responder by month. A monthly responder is a CSBM weekly responder for at least 3 of the 4 treatment period weeks for that month. A subject with missing response at a specific month was considered a non-responder for that month.

For Study LIN-MD-01, greater proportions of monthly responders in the linaclotide group were observed for each month of the study. The linaclotide 266 µg dose group showed numerically higher response rates than the linaclotide 133 µg group from Month 1 through Month 3. But, the dose group difference decreased to 5.7% by Month 3.

Contrary to the finding from Study LIN-MD-01, Study MCP-103-303 showed that the monthly responder rates for the linaclotide 266 µg was numerically lower than those for the linaclotide 133 µg for each month of the study. At Month 3, the linaclotide 266 µg was 6% less than that for linaclotide 133 µg.

To assess sustained efficacy, this reviewer performed post-hoc analyses using a more stringent definition of 12-week overall responder. A subject was considered to be a responder if the subject was a monthly responder for all 3 months. A subject was considered to be a monthly responder if the subject was a weekly responder for at least 3 out of 4 weeks in the month.

For this more stringent definition, both linaclotide doses (133 µg and 266 µg) were superior to the placebo in both studies. Treatment differences were 7.5% and 12.8%, for linaclotide 133 µg and 266 µg, respectively in Study LIN-MD-01. Similar treatment differences were observed in Study MCP-103-303 (10.0% and 9.1%).

To assess sustained efficacy, this reviewer also performed post-hoc analyses using another more stringent definition of overall responder. A subject was considered to be

a responder if the subject was a 12-week overall responder and was a weekly responder for at least 3 of 4 weeks in Month 3.

For this responder definition, both linaclotide doses (133 µg and 266 µg) were superior to the placebo in both studies. Treatment differences were 9.9% and 12.0%, for linaclotide 133 µg and 266 µg, respectively in Study LIN-MD-01. Similar treatment differences were observed in Study MCP-103-303 (13.2% and 14.2%).

In conclusion, both studies (MCP-103-303 and LIN-MD-01) showed that both linaclotide doses (133 µg and 266 µg) were superior to the placebo for protocol-specified primary efficacy endpoint.

In review of safety, this reviewer found that greater proportions of subjects with adverse events in the linaclotide group compared with subjects in the placebo group for both studies (MCP-103-303 and LIN-MD-01). These included proportions of subjects with at least one treatment related AE (TRAE), withdrew due to AE, with at least one episode of diarrhea, or discontinued due to TRAE of diarrhea.

2. INTRODUCTION

2.1 Overview

Linaclotide is a minimally absorbed 14-amino-acid peptide that acts locally in the intestinal lumen to stimulate the guanylate cyclase subtype C (GC-C) receptor. By activating the GC-C receptor, orally administered linaclotide has been found in animal models to increase intestinal fluid secretion and intestinal transit, and also to decrease visceral pain.

Linaclotide, a 14-amino acid synthetic peptide, is a potent and selective GC-C receptor agonist structurally related to the endogenous guanylin peptide family. Activation of the GC-C receptor results in an increase in both intracellular and extracellular concentrations of cyclic guanosine monophosphate (cGMP). Elevation in intracellular cGMP stimulates secretion of chloride and bicarbonate into the intestinal lumen, through activation of the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel, resulting in increased intestinal fluid and accelerated transit. Extracellular cGMP decreases the activity of pain-sensing nerves, which is thought to be responsible for the observed reduction in visceral pain.

The sponsor seeks marketing approval for linaclotide as an orally administered treatment for irritable bowel syndrome with constipation (IBS-C) and chronic idiopathic constipation (CIC).

2.2 Data Sources

The sponsor has submitted two adequate and well-controlled studies (MCP-103-302) and LIN-MD-31) for the irritable bowel syndrome with constipation (IBS-C) indication and two adequate and well-controlled studies (MCP-103-303) and LIN-MD-01) for the chronic idiopathic constipation (CIC) indication.

The four pivotal studies are

MCP-103-302: A Phase 3, Randomized, Double-blind, Placebo-controlled, Parallel-group trial of Linaclotide Administered Orally for 26 weeks in Patients with Irritable Bowel Syndrome with Constipation

LIN-MD-31: A Phase 3, Randomized, Double-blind, Placebo-controlled, Parallel-group trial of Linaclotide Administered Orally for 12 weeks Followed by a 4-Week Randomized Withdrawal Period in Patients with Irritable Bowel Syndrome with Constipation

LIN-MD-01: A Phase 3, Randomized, Double-blind, Placebo-controlled, Parallel-group trial of Linaclotide Administered Orally for 12 weeks in Patients with Chronic Constipation

MCP-103-303: A Phase 3, Randomized, Double-blind, Placebo-controlled, Parallel-group trial of Linaclotide Administered Orally for 12 weeks Followed by a 4-Week Randomized Withdrawal Period in Patients with Constipation

This review will focus on the two studies (MCP-103-303 and LIN-MD-01) for the chronic idiopathic constipation (CIC) indication.

The original submission was submitted in eCTD and dated August 9, 2011.

The electronic submission is located at \\Cdsesub1\evsprod\NDA202811\0000.

The sponsor submitted responses to requests for information dated November 30, 2011, December 22, 2011, January 20, 2012, January 30, 2012, February 8, 2012, March 5, 2012, April 10, 2012, April 16, 2012, April 18, 2012, April 19, 2012, May 2, 2012 and May 24 2012.

3. STATISTICAL EVALUATION

3.1 Evaluation of Efficacy

3.1.1 Study LIN-MD-01

3.1.1.1 Study Design

This is a phase 3, randomized, double-blind, placebo-controlled, parallel-group trial of Linaclotide administered orally for 12- weeks trial comparing 2 doses of

linaclotide with placebo in patients with chronic idiopathic constipation (modified Rome II criteria). The trial was conducted in the U.S. (95 sites) and Canada (8 sites).

The objective of this trial was to determine the efficacy and safety of linaclotide administered to patients with chronic idiopathic constipation.

The trial consisted of the following periods: up to 21 days of screening, 14 to 21 days of pretreatment, and 12 weeks of double-blind treatment. At the end of the pretreatment period, during which patients provided qualifying bowel habit, symptom severity, and rescue medicine information, patients meeting the entry criteria for this trial were randomized to 1 of 3 double-blind treatment groups: 133 μg/day linaclotide, 266 μg/day linaclotide, or placebo (1:1:1).

The Screening Period (Visit 1) started when the patient signed the ICF and lasted for up to 21 calendar days. During this period, patient eligibility for entry into the pretreatment period was determined. The end of the screening period coincided with the start of the pretreatment period. Any over-the-counter or prescription laxatives, suppositories, or enemas used to treat CC were not to be taken during the calendar day before the Pretreatment Visit (Visit 2), whereas other prohibited medicines were not to be taken during the 14 calendar days before the Pretreatment Visit.

The pretreatment period is defined as the 14 calendar days (minimum) to 21 calendar days (maximum) immediately before randomization. During this period, patients had to provide the following information through daily IVRS calls:

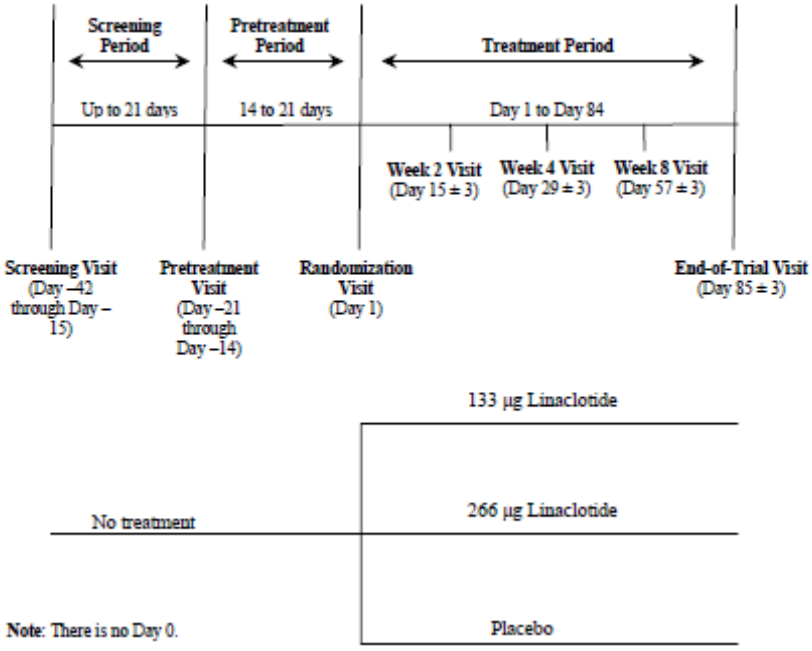
- Daily bowel habits and daily patient symptom severity assessments
- Weekly patient assessment of constipation severity
- Weekly patient assessment of degree of relief of constipation symptoms
- Use of per-protocol rescue medicine or any other laxatives, suppositories, or enemas

The treatment period began with randomization and lasted for 12 weeks. Patients who met all entry criteria were randomized to treatment with 133 μg linaclotide, 266 μg linaclotide, or placebo (1:1:1). Except for the first dose, study drug was to be taken once daily in the morning at least 30 minutes before breakfast. Patients had to take their initial dose of study drug at the trial center during the Randomization Visit on Day 1 (Visit 3). Patients must have fasted for at least 2 hours before arriving at the clinic for the Randomization Visit (Visit 3) and End-of-Trial (EOT) Visit (Visit 7). Patients were not to take any study drug on the day of the EOT Visit (Visit 7). Patients were to continue to call the IVRS to provide their daily assessments (daily bowel habit assessments and daily patient symptom-severity assessments), their weekly assessments (patient assessment of constipation severity and patient assessment of degree of relief of constipation symptoms), and their use of rescue medicine and any other laxatives, suppositories, or enemas. A Treatment Satisfaction Assessment was to be performed at the Week 2 Visit (Visit 4) and all subsequent visits. A Treatment Continuation Assessment was to be performed at the EOT Visit

(Visit 7). A number of quality-of-life and patient-outcome assessments were performed at trial visits throughout the treatment period.

The overview of trial design is given below.

Figure 9.1.3–1. Overview of Trial Design



Main inclusion criteria are:

1. Males and females ≥ 18 years of age were included if they
2. Meet Rome II criteria for CC, which have been slightly modified from the original: patient reported fewer than 3 bowel movements (BMs) per week (with each BM occurring in the absence of any laxative, suppository, or enema use during the preceding 24 hours) and reported 1 or more of the following symptoms for at least 12 weeks, which need not be consecutive, in the 12 months before the Screening Visit (Visit 1) or before starting chronic treatment with tegaserod, lubiprostone, polyethylene glycol 3350, or any laxative:
   a. Straining during more than 25% of BMs
   b. Lumpy or hard stools during more than 25% of BMs
   c. Sensation of incomplete evacuation during more than 25% of BMs
3. Report an average of fewer than 3 CSBMs per week and 6 or fewer SBMs per week by the IVRS during the 14 days before the start of the treatment period.
4. Be compliant with IVRS completion by adequately responding to IVRS questions on 10 or more of the 14 days before the start of the treatment period

Patients were excluded for any of the following reasons:

1. They reported a Bristol Stool Form Scale (BSFS) score of 6 (loose, mushy stools) for more than 1 SBM or a BSFS score of 7 (watery stool) with any SBM during the 14 days before the start of the Treatment Period;
3. They used Rescue Medicine (bisacodyl tablet or suppository) or any other laxative, suppository, or enema on the calendar day before or the calendar day of the start of the Treatment Period.

The primary efficacy assessment, which was used to determine the primary efficacy parameter (12-week CSBM overall responder) during the 12 weeks, was based the IVRS information that determined whether a BM was a CSBM.

Each day of the pretreatment and treatment periods, the patient called the IVRS and provided the number of BMs he or she had since the previous day's call. Patients were only allowed to call between the hours of 12 noon and 11:59 PM, and they were asked to call at about the same time each day. For each BM, the patient also provided the day the BM occurred and if the BM was associated with a sense of complete evacuation (the patient was also asked to provide assessments of consistency and straining, which are secondary efficacy assessments). The patient was also asked if he or she took any medicines to treat his or her constipation since the previous day's call. For each type of rescue medicine taken (e.g., bisacodyl tablet, bisacodyl suppository) or other laxatives, suppositories, or enemas, the patient was asked to provide the day it was taken.

In addition to the primary efficacy assessment, the following efficacy assessments were used in determining the secondary efficacy parameters:

The SBM assessment was based on the IVRS information that determined whether a BM was an SBM as defined for the primary efficacy parameter.

Patient assessment of stool consistency was collected daily by IVRS calls. For each BM, stool consistency was assessed by the patient using the BSFS. The 7-point ordinal BSFS scale is provided below:
"Please describe the consistency of the bowel movement using the following scale where:"

1=Separate hard lumps like nuts (difficult to pass)
2=Sausage shaped but lumpy
3=Like a sausage but with cracks on surface
4=Like a sausage or snake, smooth and soft
5=Soft blobs with clear-cut edges (passed easily)
6=Fluffy pieces with ragged edges, a mushy stool
7=Watery, no solid pieces (entirely liquid)

Patient assessment of straining was collected daily by IVRS calls. For each BM, degree of severity of straining was assessed by the patient using the following 5-point ordinal scale:

"How much did you strain during the bowel movement?"

1=Not at all
2=A little bit
3=A moderate amount
4=A great deal
5=An extreme amount

Patient assessment of constipation severity was reported weekly by IVRS calls. The rating of constipation severity during the previous 7 days on a 5-point ordinal scale was provided by the patient answering the following question:
"On average, how would you rate your constipation during the past 7 days?"

1 = none
2 = mild
3 = moderate
4 = severe
5 = very severe

Patient assessment of abdominal discomfort was collected daily by IVRS calls. The rating of abdominal discomfort during the previous 24 hours on a 5-point ordinal scale was provided by the patient answering the following question:
"How would you rate your abdominal discomfort over the last 24 hours?"

1 = none
2 = mild
3 = moderate
4 = severe
5 = very severe

Patient assessment of bloating was collected daily by IVRS calls. The rating of bloating during the previous 24 hours on a 5-point ordinal scale was provided by the patient answering the following question:
"How would you rate your bloating over the last 24 hours?"

1 = none
2 = mild
3 = moderate
4 = severe
5 = very severe

In addition to the primary and secondary efficacy assessments, the following efficacy assessments were used in determining the additional efficacy parameters.

This assessment is the IVRS question asking the patient if a BM occurred within 24 hours of the patient receiving the first dose of study drug.
"Did this bowel movement occur less than 24 hours after you first took study medication?"

1 = yes
2 = no

Patient assessment of abdominal pain was collected daily by IVRS calls. The rating of abdominal pain during the previous 24 hours on a 5-point ordinal scale was provided by the patient answering the following question:
"How would you rate your abdominal pain over the last 24 hours?"

1 = none
2 = mild
3 = moderate
4 = severe
5 = very severe

Patient assessment of degree of relief of constipation symptoms was reported weekly by IVRS calls. The rating on a 7-point balanced ordinal scale was provided by the patient answering the following question:
"Compared to before you started this study, how would you rate your constipation symptoms during the past 7 days?"

1 = completely relieved
2 = considerably relieved
3 = somewhat relieved
4 = unchanged
5 = somewhat worse
6 = considerably worse
7 = as bad as I can imagine

A treatment-satisfaction assessment was performed at Week 2 (Visit 4) and at all subsequent trial visits. Patients answered the following 5-point ordinal scale question:
"Overall, how satisfied are you with the study medication's ability to relieve your constipation symptoms?"

1 = not at all satisfied
2 = a little satisfied
3 = moderately satisfied
4 = quite satisfied
5 = very satisfied

A treatment-continuation assessment was performed at the EOT Visit (Visit 7). Patients answered the following 5-point ordinal scale question:
"If given the option, how likely is it that you would continue taking the study medication?"

1 = not at all likely
2 = a little likely
3 = moderately likely
4 = quite likely
5 = very likely

Four populations were considered in the statistical analysis of the study.

The Screened Population consisted of all patients who had a Screening Visit (Visit 1) and were assigned a PID number.

The Randomized Population consisted of all patients in the Screened Population who were randomized to a treatment group at the Randomization Visit (Visit 3).

The Safety Population consisted of all patients in the Randomized Population who received at least 1 dose of double-blind study medication during the treatment period.

The Intent-to-Treat (ITT) Population consisted of all patients in the Safety Population who had at least 1 postrandomization entry of the primary efficacy assessment (i.e., the daily IVRS information that determined whether an SBM is a CSBM).

3.1.1.2 Sponsor's Analysis

Of the total of 1232 patients screened, 633 were randomized to treatment and received at least 1 dose of study drug. All but 3 of the patients were included in the ITT Population (See table below.) The US southeast region had the greatest number of randomized patients (N = 294).

Patient Populations
LIN-MD-01

Patients screened = 1232				
Screen failures = 274				
Pretreatment failures = 325				
	Placebo	*Linaclotide*		*Total*
		133 µg/day	*266 µg/day*	
Patients randomized	215	213	205	633
Safety Population	215	213	205	633
Intent-to Treat Population	215	213	202	630

Data source: Table 14.1.1 and Table 14.1.2.

Of the 1232 screened patients, there were 274 (22.2%) screen failures and 325 (26.4%) pretreatment failures (i.e., patients who entered the pretreatment period but were not randomized). Most of these patients did not meet the inclusion/exclusion criteria for randomization.

Table below summarizes the disposition data. A significantly greater percentage of patients in the linaclotide 133-µg/day group discontinued from the study than did the placebo patients (18.8% vs. 11.2%; p = 0.0303). Most of the discontinuations in each treatment group were the result of an AE; the discontinuation rate due to an AE was significantly greater in the linaclotide 133-µg/day group than in the placebo group (9.9% vs. 4.7%; p = 0.0413). In addition, more linaclotide 133-µg/day patients were lost to follow-up than were placebo patients (4.2% vs. 0.5%; p = 0.0106). No other treatment group comparisons were statistically significant.

Number (%) of Patients Discontinued During Treatment Period
Randomized Population
LIN-MD-01

	Placebo (N = 215)	*Linaclotide*		*Total (N = 633)*
		133 µg/day (N = 213)	*266 µg/day (N = 205)*	
	n (%)	n (%)	n (%)	n (%)
Completed study	191 (88.8)	173 (81.2)	169 (82.4)	533 (84.2)
Discontinued from study	24 (11.2)	40 (18.8)	36 (17.6)	100 (15.8)
Reason for discontinuation				
Adverse event	10 (4.7)	21 (9.9)	20 (9.8)	51 (8.1)
Insufficient therapeutic response	4 (1.9)	0	1 (0.5)	5 (0.8)
Protocol violation	4 (1.9)	3 (1.4)	1 (0.5)	8 (1.3)
Withdrawal of consent	2 (0.9)	6 (2.8)	6 (2.9)	14 (2.2)
Lost to follow-up	1 (0.5)	9 (4.2)	6 (2.9)	16 (2.5)
Other	3 (1.4)	1 (0.5)	2 (1.0)	6 (0.9)

Data source: Table 14.1.3.

3.1.1.2.1 Planned Analysis

The primary efficacy parameter was 12-week CSBM overall responder. A 12-week CSBM overall responder was a patient who was a CSBM weekly responder for at least 9 of the 12 weeks of the treatment period. A CSBM weekly responder was a patient who had a CSBM weekly frequency rate that was 3 or greater and increased by 1 or more from baseline. If a patient did not have CSBM frequency data for a particular week of the treatment period, the patient was not considered a CSBM weekly responder for that week.

If a patient prematurely discontinued from trial, and the patient's final treatment period week contained less than 4 days, the patient was not considered a CSBM weekly responder for that week or the subsequent missed weeks of the treatment period.

There were 7 secondary efficacy parameters:

1. Change from Baseline in 12-week CSBM Frequency Rate,
2. Change from Baseline in.12-week SBM Frequency Rate,
3. Change from Baseline in 12-week Stool Consistency,
4. Change from Baseline in 12-week Severity of Straining,
5. Change from Baseline in 12-week Abdominal Discomfort,
6 Change from Baseline in 12-week Bloating,
7 Change from Baseline in.12-week Constipation Severity

The role of the additional efficacy parameters was to provide additional support for the primary and secondary efficacy parameters.

Additional efficacy parameters are:

1. BM within 24 hours of receiving the first dose of study drug,
2. Change from Baseline in 12-week abdominal pain,
3. Complete Spontaneous Bowel Movement Weekly Responder,
4. Degree of relief of constipation symptom responder,
5. Treatment satisfaction,
6. Treatment constipation.

Demographic parameters (i.e., age, race, sex, weight, height, and body mass index) and other baseline characteristics, including baseline efficacy measurements, were summarized by treatment group for the Safety and ITT populations. Comparability among treatment groups was tested using a 2-way analysis-of-variance (ANOVA) model with treatment group and geographic region as the factors for continuous variables. A Cochran-Mantel-Haenszel (CMH) test, controlling for geographic region, was used for categorical variables.

All efficacy analyses were based on the ITT Population.

Spontaneous bowel movement was defined as a BM that occurred in the absence of laxative, enema, or suppository use on either the calendar day of the BM or the calendar day before the BM. CSBM was defined as an SBM that was associated with a sense of complete evacuation.

Baseline values for efficacy parameters were derived from the IVRS daily diary collected in the pretreatment period, specifically the period of time from 14 days before randomization up to the time of randomization. The baseline CSBM and SBM weekly rates were derived as the corresponding overall weekly frequency rates based on the number of CSBMs and SBMs a patient had during this period. Baseline stool consistency and severity of straining were calculated as the average of the nonmissing values from the SBMs reported by the patient during this period. Baseline values for patient symptom and global assessments (e.g., abdominal discomfort, bloating, abdominal pain, constipation severity, degree of relief of constipation symptoms) were the average of the nonmissing patient scores reported during this period.

An observed-cases (OC) approach was applied to missing post baseline data. In addition, a last-observation-carried-forward approach (LOCF) approach was used for sensitivity analyses for all secondary efficacy parameters that were defined on a weekly basis. In the LOCF method, a patient's last weekly value was used when the patient prematurely discontinued from the trial, or the patient's previous weekly value was used when the patient's current weekly value was missing. For efficacy analyses, trial centers were pooled together by geographic region.

The overall type I family-wise error rate for testing the primary and secondary efficacy parameters was controlled at the 0.05 significance level. All confidence intervals were 2-sided 95% confidence intervals, unless stated otherwise.

This trial was designed to test the following 2 sets of primary efficacy hypotheses:

1. *Null hypothesis*: There was no difference in the proportion of 12-week CSBM overall responders between patients taking the 266-µg dose and those taking placebo
   Alternative hypothesis: There was a difference in the proportion of 12-week CSBM overall responders between patients taking the 266-µg dose and those taking placebo
2. *Null hypothesis:* There was no difference in the proportion of 12-week CSBM overall responders between patients taking the 133-µg dose and those taking placebo
   Alternative hypothesis: There was a difference in the proportion of 12-week CSBM overall responders between patients taking the 133-µg dose and those taking placebo

The primary efficacy analysis was the CMH test controlling for geographic region. For each of the 2 linaclotide dose groups, the proportion of 12-week CSBM overall responder was compared with the proportion in the placebo group using the CMH test controlling for geographic region. The number and percentage of 12-week CSBM overall responders for each treatment group, the difference in responder rates between each linaclotide and placebo group, and the 2-sided p-value associated with the above CMH test were presented. The Mantel-Haenszel estimate of odds ratio (controlling for geographic region) and the corresponding 95% confidence interval for each linaclotide dose group over placebo group are also provided.

For each of the following secondary efficacy parameters, each of the 2 linaclotide dose groups were compared with the placebo group using an analysis-of-covariance (ANCOVA) model with fixed-effect terms for treatment group and geographic region and the patient's corresponding baseline value of the parameter as a covariate. Least squares means for each treatment group, difference in least squares means between each of the 2 linaclotide dose treatment groups versus placebo, associated 2-sided 95% confidence interval for these differences in least squares means, and the corresponding p-value were reported.

The overall type I family-wise error rate for testing the primary and secondary efficacy parameters was controlled at the 0.05 significance level using the following 5-step serial gatekeeping multiple comparison procedure (MCP). Following this MCP, progression to the next step only occurred if all individual hypotheses within a step were rejected and the previous step(s) were all rejected at the step-specific overall significance level. If all hypotheses within a step were not rejected, the hypothesis tests involved in all subsequent steps were considered not statistically significant. All hypothesis tests were 2-sided.

1. The first step tested the primary efficacy parameter for the 266-µg dose group at the 0.05 significance level

2. The second step tested the primary efficacy parameter for the 133-µg dose group and the following 5 secondary parameters for the 266-µg dose group:
   - Change from baseline in 12-week CSBM frequency
   - Change from baseline in 12-week SBM frequency
   - Change from baseline in 12-week stool consistency
   - Change from baseline in 12-week severity of straining
   - Change from baseline in 12-week constipation severity

The 6 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters within this step.

3. The third step tested the following 2 secondary efficacy parameters for the 266-µg dose group
   - Change from baseline in 12-week abdominal discomfort
   - Change from baseline in 12-week bloating

The 2 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters within this step.

4. The fourth step tested the following 5 secondary efficacy parameters for the 133-µg dose group:
   - Change from baseline in 12-week CSBM frequency
   - Change from baseline in 12-week SBM frequency
   - Change from baseline in 12-week stool consistency
   - Change from baseline in 12-week severity of straining
   - Change from baseline in 12-week constipation severity

The 5 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters within this step. (Note that this is the same set of secondary parameters tested in step 2 for the 266-µg dose group.)

5. The fifth step tested the following 2 secondary efficacy parameters for the 133-µg dose group:
   - Change from baseline in 12-week abdominal discomfort
   - Change from baseline in 12-week bloating

The 2 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters

within this step. (Note that this is the same set of secondary parameters tested in step 3 for the 266-µg dose group.)

The power calculation for the primary efficacy parameter was based on the results of Ironwood study MCP-103-201, a Phase 2b study in which 310 patients with CC were randomized to linaclotide or placebo once daily for 4 weeks. The 4-week CSBM overall responder rates for patients treated with placebo was 7.4%; the 4-week CSBM overall responder rate for patients treated with linaclotide were 18.6%, 26.8%, 32.3%, and 29.0% for the 67-µg, 133-µg, 266-µg, and 532-µg linaclotide dose groups, respectively.

For the Phase 3 primary efficacy parameter power calculations, the placebo group estimate is taken from the placebo rate (7.4%) in study MCP-103-201. The 133-µg linaclotide group estimate is based on the combination of responder rates for the 67-µg and 133-µg dose groups (22.6%), and the 266 µg linaclotide group estimate is based on the combination of responder rates for all (67 µg, 133 µg, 266 µg, and 532 µg) linaclotide dose groups (27.2%). Assuming that 15% more randomized patients would discontinue from a 12-week treatment period of this trial than from the 4-week treatment period in MCP-103-201 (20%) and not be responders, the 12-week CSBM overall responder rate estimates used in these power calculations were 6.3%, 19.2%, and 23.1%, for the placebo, 133-µg, and 266-µg groups, respectively.

Based on the above estimates of the anticipated 12-week CSBM overall responder rates from the Phase 2b data, and the MCP, a trial with 200 patients randomized to each of the 3 treatment groups would have greater than 96% power to reject the 266-µg dose group primary efficacy parameter hypothesis and at least 90% power to reject the 133-µg dose group primary efficacy parameter hypothesis.

3.1.1.2.2 Treatment Group Comparability

A summary of the results of the comparability of treatment groups at baseline for all randomized patients is given in the Appendix Tables 1 and 2.

As seen from the Appendix Table 1, demographics and baseline characteristics were comparable among treatment groups. In contrast, the mean weight of the placebo patients was somewhat higher than that of the linaclotide patients; the mean body mass index in the linaclotide 266 µg/day group was significantly less than that of the placebo patients (27.4 kg/m2 vs. 28.8 kg/m2; p = 0.0218).

As seen from the Appendix Table 2, overall, baseline efficacy parameters were similar among groups.

The most commonly used concomitant medications (≥5%) were similar among the treatment groups.

Mean compliance rates in each treatment group was greater than 90% throughout the study. Compliance rates (patients with ≥ 80% complete calls) were 72.6% for placebo patients, 75.1% for linaclotide 133-µg/day patients, and 75.7% for linaclotide 266 µg/day patients over the course of the double-blind treatment period.

3.1.1.2.3 Sponsor's Analysis of Primary Efficacy Parameter

The primary efficacy endpoint was the number of patients who were 12-week CSBM overall responders, defined as patients who were CSBM responders for at least 9 of the 12 weeks of the treatment period; a CSBM weekly responder was a patient who had a CSBM weekly frequency rate that was 3 or greater and increased by 1 or more from baseline.

The results from the analysis of the 12-week CSBM overall responders in the ITT population are given below.

Primary Efficacy Analyses: 12-Week CSBM Overall Responders
ITT Population
LIN-MD-01

	Placebo (N = 215)	*Linaclotide*	
		133 µg/day (N = 213)	*266 µg/day (N = 202)*
Responder, n (%)	13 (6.0)	34 (16.0)	43 (21.3)
Nonresponder, n (%)	202 (94.0)	179 (84.0)	159 (78.7)
Difference in responder rate (linaclotide – placebo)	—	9.9	15.2
Odds ratio (95% CI)	—	2.93 (1.50, 5.72)	4.22 (2.20, 8.10)
p-Value	—	0.0012	< 0.0001

A 12-week CSBM overall responder was a patient who was a CSBM weekly responder for at least 9 of the 12 weeks of the double-blind treatment period.

Odds ratios were estimated using the Mantel-Haenszel method controlling for geographic region.

p-Values were obtained from the Cochran-Mantel-Haenszel tests controlling for geographic region, comparing each linaclotide dose versus placebo in a pairwise manner.

Both p-values met the criterion for statistical significance based on the multiple comparison procedure.

CI = confidence interval; CSBM = complete spontaneous bowel movement; ITT = intent to treat; N = population size; n = number of responders within a group.

Data source: Table 14.4.1.1

As seen from the table above, the number and percentage of patients who were 12-Week CSBM overall responders were greater for each linaclotide group when compared to placebo.

3.1.1.2.4 CSBM Weekly Responders and Improvement in 12-Week CSBM Rate at Incremental Levels

The sponsor also performed analysis of CSBM weekly responders by week. The percentage of patients who were CSBM weekly responders are presented graphically

below as supportive to the primary efficacy parameter. Discontinued patients were considered CSBM nonresponders for those weeks subsequent to their discontinuation.

Percent CSBM Weekly Responders—ITT Population
LIN-MD-01
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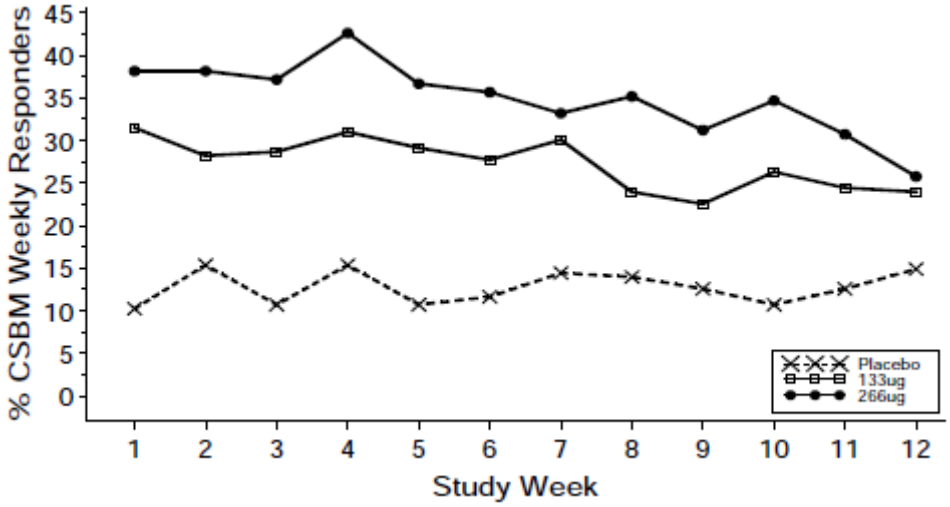

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As seen from the figure above, during each week of the treatment period, the proportion of patients who were CSBM weekly responders (patients who had ≥ 3 CSBMs and a change from baseline of ≥1 during the particular week) was greater with each linaclotide dose group than with placebo.

3.1.1.2.5 Sponsor's Analyses of Secondary Variables

The secondary efficacy parameters based on the IVRS calls were:

- Change from baseline in 12-week CSBM frequency rate
- Change from baseline in 12-week SBM frequency rate
- Change from baseline in 12-week stool consistency
- Change from baseline in 12-week severity of straining
- Change from baseline in 12-week abdominal pain
- Change from baseline in 12-week abdominal discomfort
- Change from baseline in 12-week bloating
- 12-Week Constipation Severity

3.1.1.2.5.1 Change from Baseline in 12-week CSBM Frequency Rate

A summary of the results of the analysis of the change from baseline in 12-week CSBM frequency rate for treatment overall is given below.

Change From Baseline in 12-Week CSBM Frequency Rate
ITT Population
LIN-MD-01

Visit	*Statistic*	*Placebo*	*Linaclotide*	
		N = 215	133 µg/day (N = 213)	266 µg/day (N = 202)
Baseline	Mean	0.274	0.261	0.276
	SD	0.517	0.505	0.552
	SEM	0.035	0.035	0.039
	Median	0.000	0.000	0.000
	Min, max	0.00, 1.95	0.00, 2.43	0.00, 2.43
	n	215	213	202
Treatment overall	Mean	0.903	2.246	2.936
	SD	1.283	2.956	3.688
	SEM	0.088	0.203	0.259
	Median	0.323	1.290	1.823
	Min, max	0,00, 6.54	0.00, 15.07	0.00, 29.58
	n	215	213	202
ANCOVA results	LSMC from baseline (SE)	0.614 (0.209)	2.011 (0.215)	2.653 (0.217)
	LSMD (95% CI)	—	1.397 (0.89, 1.91)	2.040 (1.52, 2.56)
	p-Value[a]	—	< 0.0001	< 0.0001

a p-Values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. p-Values are less than the threshold value for statistical significance based on the multiple comparison procedure.

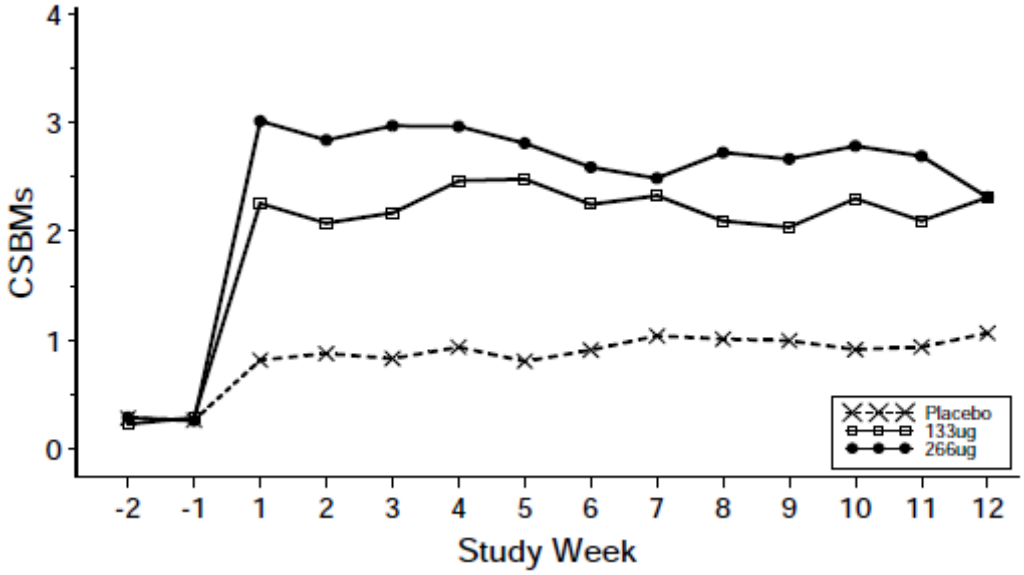
ANCOVA = analysis of covariance; CI = confidence interval; CSBM = complete spontaneous bowel movement; ITT = intent to treat; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Data source: Table 14.4.2.1A.

As seen from the table above, the difference between each linaclotide dose groups and placebo was statistically significant.

Mean CSBM frequency rates during the treatment period is plotted by week are given below.

Mean CSBM Rate (OC) by Week (Treatment Period)
ITT Population
LIN-MD-01



Copied from Figure 11.4.1.3.1-1.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.1.2.5.2 Change from Baseline in 12-week SBM Frequency Rate

A summary of the results of the analysis of the change from baseline in 12-week SBM frequency rate for treatment overall is given below.

Change From Baseline in 12-Week SBM Frequency Rate
ITT Population
LIN-MD-01

Visit	*Statistic*	*Placebo*	*Linaclotide*	
		N = 215	133 µg/day (N = 213)	266 µg/day (N = 202)
Baseline	Mean	1.815	1.846	1.942
	SD	1.427	1.501	1.550
	SEM	0.097	0.103	0.109
	Median	1.452	1.461	1.912
	Min, max	0.00, 6.20	0.00, 7.18	0.00, 6.35
	n	215	213	202
Treatment overall	Mean	2.967	5.286	5.649
	SD	2.643	3.993	4.466
	SEM	0.180	0.274	0.314
	Median	2.511	4.591	4.876
	Min, max	0.00, 24.23	0.00, 21.00	0.00, 31.70
	n	215	213	202
ANCOVA results	LSMC from baseline (SE)	1.113 (0.265)	3.466 (0.272)	3.675 (0.275)
	LSMD (95% CI)	—	2.333 (1.69, 2.98)	2.562 (1.91, 3.22)
	p-Value[a]	—	< 0.0001	< 0.0001

a p-Values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. p-Values are less than the threshold value for statistical significance based on the multiple comparison procedure.

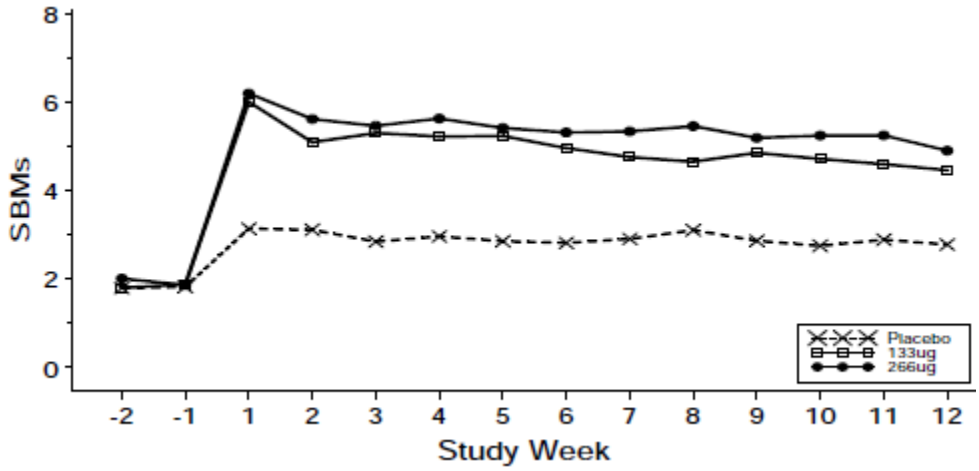
ANCOVA = analysis of covariance; CI = confidence interval; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point; SBM = spontaneous bowel movement;

Data source: Table 14.4.2.2A.

As seen from the table above, the difference between each linaclotide dose groups and placebo was statistically significant.

Mean SBM frequency rates during the treatment period are graphed below by week.

Mean SBM Rate (OC) by Week (Treatment Period)
ITT Population
LIN-MD-01



Copied from Figure 11.4.1.3.2-1.

As seem from the figure above, both the 133- and 266-µg/day doses demonstrated a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.1.2.5.2 Change from Baseline in 12-week Stool Consistency

A summary of the results of the analysis of the change from baseline in 12-week stool consistency for treatment overall is given below.

Change From Baseline in 12-Week Stool Consistency
ITT Population
LIN-MD-01

Visit	*Statistic*	*Placebo*	*Linaclotide*	
		N = 215	133 µg/day (N = 213)	266 µg/day (N = 202)
Baseline	Mean	2.345	2.352	2.335
	SD	1.025	1.059	1.053
	SEM	0.076	0.078	0.081
	Median	2.250	2.268	2.162
	Min, max	1.00, 5.00	1.00, 6.00	1.00, 5.00
	n	183	182	168
Treatment overall	Mean	2.939	4.184	4.371
	SD	0.986	1.327	1.295
	SEM	0.073	0.098	0.100
	Median	2.919	4.149	4.483
	Min, max	1.00, 5.53	1.00, 6.87	1.00, 6.85
	n	183	182	168
ANCOVA results	LSMC from baseline (SE)	0.572 (0.098)	1.823 (0.100)	2.009 (0.103)
	LSMD (95% CI)	—	1.251 (1.02, 1.49)	1.437 (1.20, 1.68)
	p-Value[a]	—	< 0.0001	< 0.0001

a p-Values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. p-Values are less than the threshold value for statistical significance based on the multiple comparison procedure.

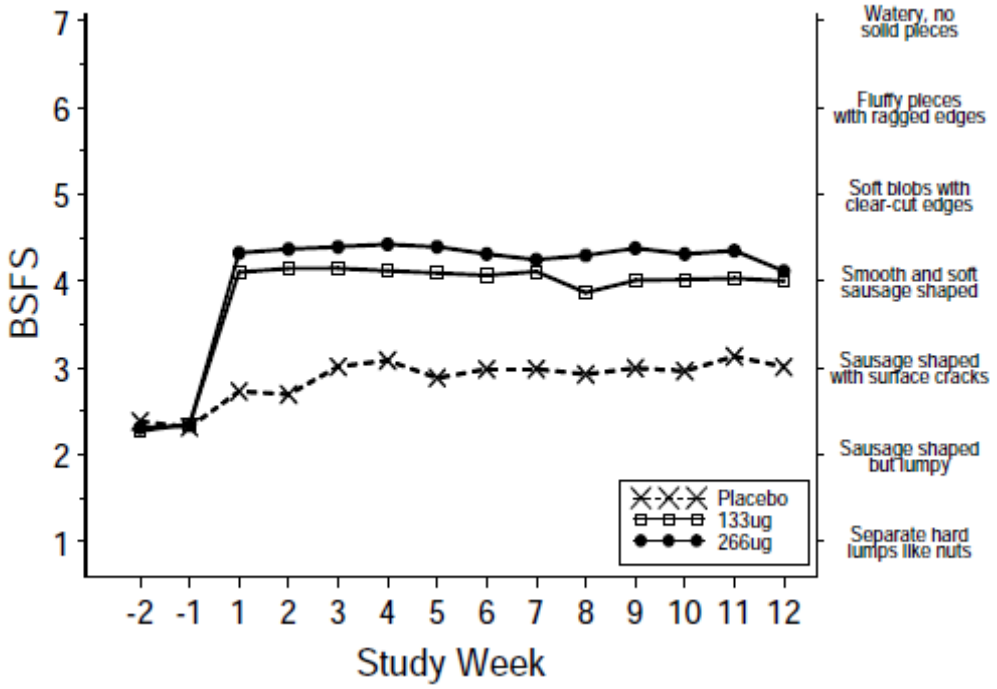
ANCOVA = analysis of covariance; CI = confidence interval; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Data source: Table 14.4.2.3A.

As seen from the table above, patients on linaclotide demonstrated a change in stool consistency to just over 4, which is in the normal range (3-5); in contrast patients on placebo showed minimal improvement to an average score of 2.9, just below the lower limit of the normal range. The differences between linaclotide and placebo patients were statistically significant.

Mean stool consistency during the treatment period is graphed below by week.

Mean Stool Consistency (OC) by Week (Treatment Period)
ITT Population
LIN-MD-01



Copied from Figure 11.4.1.3.3-1.

As seem from the figure above, both the 133- and 266-µg/day doses demonstrated a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.1.2.5.4 Change from Baseline in 12-week Severity of Straining

A summary of the results of the analysis of the change from baseline in 12-week severity of straining for treatment overall is given below.

Change From Baseline in 12-Week Severity of Straining
ITT Population
LIN-MD-01

Visit	*Statistic*	*Placebo*	*Linaclotide*	
		N = 215	133 µg/day (N = 213)	266 µg/day (N = 202)
Baseline	Mean	3.229	3.231	3.300
	SD	0.794	0.912	0.815
	SEM	0.059	0.068	0.063
	Median	3.000	3.268	3.286
	Min, max	1.00, 5.00	1.00, 5.00	1.00, 5.00
	n	183	182	168
Treatment overall	Mean	2.688	2.098	2.049
	SD	0.749	0.721	0.754
	SEM	0.055	0.053	0.058
	Median	2.625	2.026	1.928
	Min, max	1.1, 5.00	1.00, 4.06	1.00, 5.00
	n	183	182	168
ANCOVA results	LSMC from baseline (SE)	–0.554 (0.060)	–1.141 (0.061)	–1.208 (0.063)
	LSMD (95% CI)	—	–0.587 (–0.73, –0.44)	–0.654 (–0.80, –0.51)
	p-Value[a]	—	< 0.0001	< 0.0001

a p-Values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. p-Values are less than the threshold value for statistical significance based on the multiple comparison procedure.

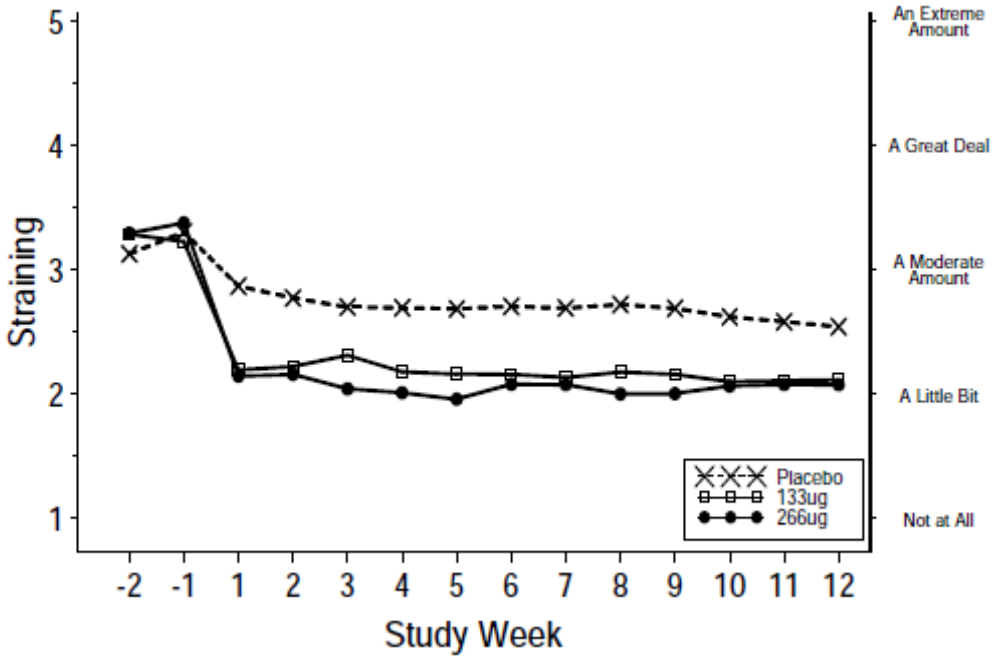
ANCOVA = analysis of covariance; CI = confidence interval; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Data source: Table 14.4.2.4A.

As seen from the table above, the linaclotide patients had a decrease in straining from about 3.2 at baseline (moderate straining) to about 2.1 (little straining). The placebo patients had a less robust decrease in straining from about 3.2 at baseline to 2.7. The differences between linaclotide and placebo patients were statistically significant.

Mean severity of straining during the treatment period is graphed below by week.

Mean Severity of Straining (OC) by Week (Treatment Period)
ITT Population
LIN-MD-01



Copied from Figure 11.4.1.3.4.1-1.

As seem from the figure above, both the 133- and 266-µg/day doses demonstrated a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.1.2.5.5 Change from Baseline in 12-week Abdominal Discomfort

A summary of the results of the analysis of the change from baseline in 12-week abdominal discomfort for treatment overall is given below.

Change From Baseline in 12-Week Abdominal Discomfort
ITT Population
LIN-MD-01

Visit	*Statistic*	*Placebo*	*Linaclotide*	
		N = 215	133 µg/day (N = 213)	266 µg/day (N = 202)
Baseline	Mean	2.557	2.468	2.515
	SD	0.842	0.858	0.903
	SEM	0.057	0.059	0.064
	Median	2.667	2.385	2.467
	Min, max	1.00, 5.00	1.00, 4.60	1.00, 4.80
	n	215	213	201
Treatment overall	Mean	2.253	2.011	2.008
	SD	0.803	0.741	0.800
	SEM	0.055	0.051	0.056
	Median	2.286	1.955	1.887
	Min, max	1.00, 4.83	1.00, 4.62	1.00, 4.64
	n	215	213	201
ANCOVA results	LSMC from baseline (SE)	–0.271 (0.043)	–0.455 (0.044)	–0.485 (0.045)
	LSMD (95% CI)	—	–0.185 (–0.29, –0.08)	–0.215 (–0.32, –0.11)
	p-Value[a]	—	0.0006	< 0.0001

a p-Values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. p-Values are less than the threshold value for statistical significance based on the multiple comparison procedure.

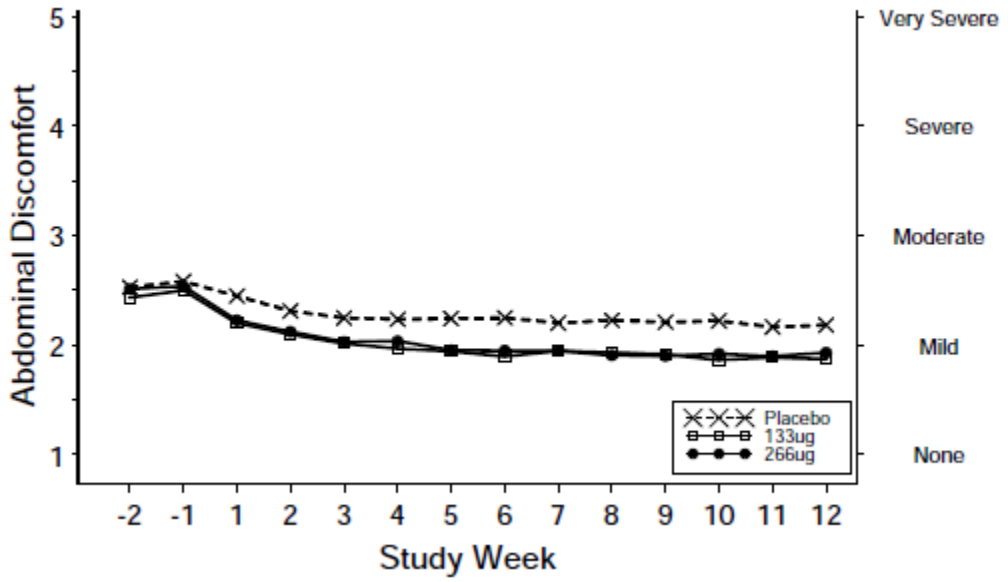
ANCOVA = analysis of covariance; CI = confidence interval; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Data source: Table 14.4.2.6A.

As seen from the table above, the linaclotide patients had a decrease in abdominal discomfort from about 2.5 at baseline (mild to moderate discomfort) to about 2.0 (mild discomfort). The placebo patients had a somewhat smaller decrease in abdominal discomfort (from about 2.6 at baseline to 2.3). The differences between linaclotide and placebo patients were statistically significant.

Mean abdominal discomfort during the treatment period is graphed below by week.

Mean Abdominal Discomfort (OC) by Week (Treatment Period)
ITT Population
LIN-MD-01



Copied from Figure 11.4.1.3.5.1-1.

As seen from the figure above, both the 133- and 266-µg/day doses demonstrated a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.1.2.5.6 Change from Baseline in 12-week Bloating

A summary of the results of the analysis of the change from baseline in 12-week bloating is given below.

Change From Baseline in 12-Week Bloating
ITT Population
LIN-MD-01

Visit	*Statistic*	*Placebo*	*Linaclotide*	
		N = 215	133 µg/day (N = 213)	266 µg/day (N = 202)
Baseline	Mean	2.815	2.776	2.729
	SD	0.873	0.844	0.951
	SEM	0.060	0.058	0.067
	Median	2.929	2.800	2.786
	Min, max	1.00, 5.00	1.00, 5.00	1.00, 4.93
	n	215	213	201
Treatment overall	Mean	2.579	2.345	2.257
	SD	0.880	0.835	0.892
	SEM	0.060	0.057	0.063
	Median	2.517	2.288	2.122
	Min, max	1.00, 4.97	1.00, 4.86	1.00, 4.63
	n	215	213	201
ANCOVA results	LSMC from baseline (SE)	–0.244 (0.048	–0.432 (0.049)	–0.485 (0.049
	LSMD (95% CI)	—	–0.209 (–0.33, –0.09)	–0.261 (–0.38, –0.14)
	p-Value[a]	—	0.0005	< 0.0001

a p-Values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. p-Values are less than the threshold value for statistical significance based on the multiple comparison procedure.

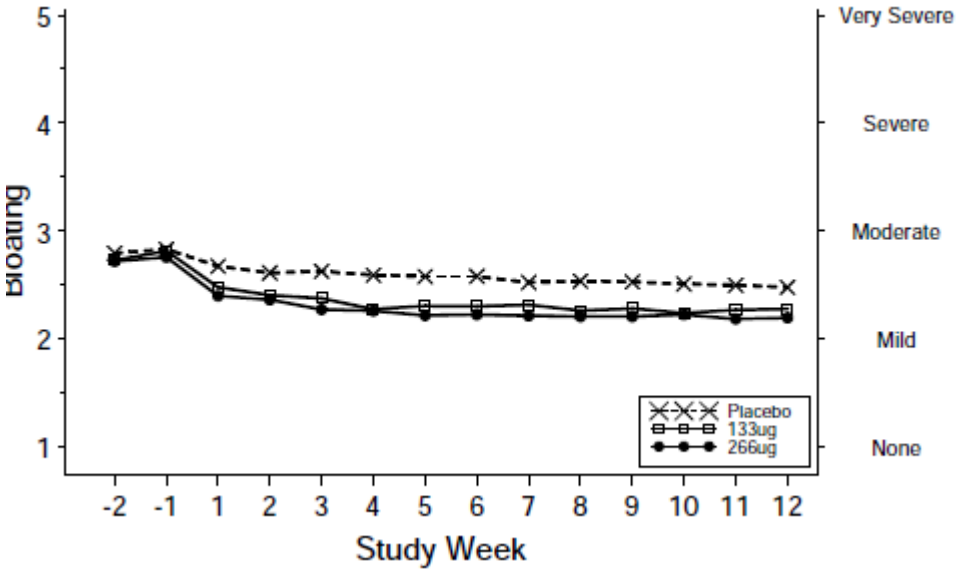
ANCOVA = analysis of covariance; CI = confidence interval; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Data source: Table 14.4.2.7A.

As seen from the table above, the linaclotide patients had a decrease in bloating from about 2.8 at baseline (moderate bloating) to about 2.3 (mild to moderate bloating). The placebo patients had a somewhat smaller decrease in bloating (from about 2.8 at baseline to 2.6). The differences between linaclotide and placebo patients were statistically significant.

Mean bloating during the treatment period is graphed below by week.

Mean Bloating (OC) by Week (Treatment Period)
ITT Population
LIN-MD-01



Copied from Figure 11.4.1.3.6-1.

As seen from the figure above, a separation from placebo was observed for all but the last week of the 12-week treatment period with the 133-µg/day linaclotide dose, and across all 12 weeks with the 266-µg/day linaclotide dose.

3.1.1.2.5.7 Change from Baseline in 12-Week Constipation Severity

A summary of the results of the analysis of the change from baseline in 12-week constipation severity for treatment overall is given below.

Change From Baseline in 12-Week Constipation Severity
ITT Population
LIN-MD-01

Visit	*Statistic*	*Placebo*	*Linaclotide*	
		N = 215	*133 µg/day (N = 213)*	*266 µg/day (N = 202)*
Baseline	Mean	3.307	3.267	3.342
	SD	0.723	0.740	0.723
	SEM	0.050	0.051	0.051
	Median	3.000	3.250	3.375
	Min, max	1.00, 5.00	1.50, 5.00	1.50, 5.00
	n	212	209	198
Treatment overall	Mean	2.976	2.356	2.343
	SD	0.815	0.850	0.867
	SEM	0.056	0.059	0.062
	Median	2.909	2.364	2.250
	Min, max	1.22, 5.00	1.00, 4.82	1.00, 4.92
	n	212	209	198
ANCOVA results	LSMC from baseline (SE)	–0.306 (0.062)	–0.908 (0.063)	–0.954 (0.064)
	LSMD (95% CI)	—	–0.602 (–0.75, –0.45)	–0.648 (–0.80, –0.49)
	p-Value[a]	—	< 0.0001	< 0.0001

a p-Values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. p-Values are less than the threshold value for statistical significance based on the multiple comparison procedure.

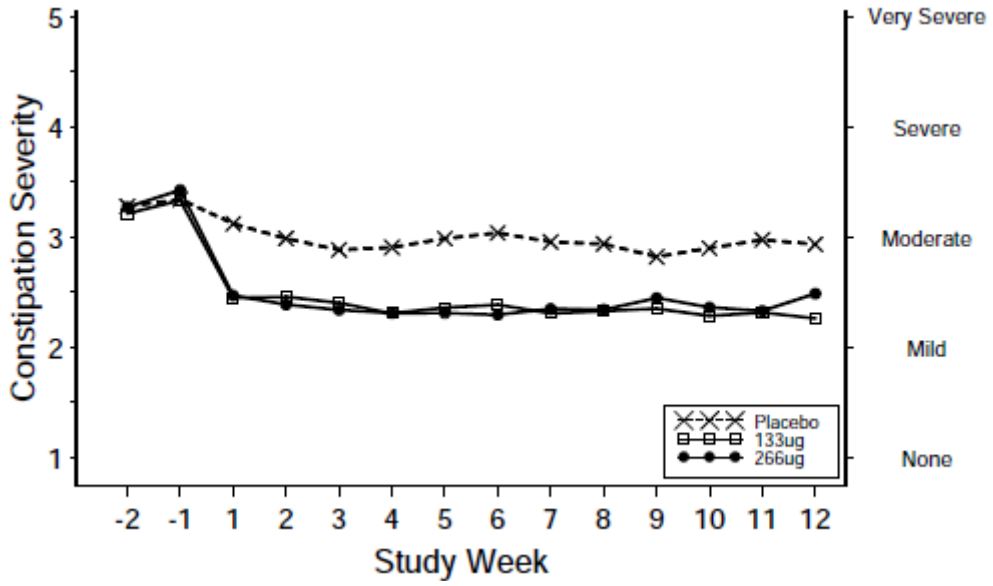
ANCOVA = analysis of covariance; CI = confidence interval; LSMC = least squares mean change; LSMD = least squares mean difference (relative to placebo); max = maximum; min = minimum; N = population size; n = number of patients with analysis values at both baseline and a specific time point.

Data source: Table 14.4.2.5A.

As seen from the table above, the linaclotide patients had a decrease in constipation severity from about 3.3 at baseline (moderate severity) to about 2.3 (mild severity). The placebo patients had a relatively slight decrease in constipation severity from about 3.3 at baseline to 3.0. The differences between linaclotide and placebo patients were statistically significant.

Mean constipation severity during the treatment period is graphed below by week.

Mean Constipation Severity (OC) by Week (Treatment Period)
ITT Population
LIN-MD-01



Copied from Figure 11.4.1.3.7-1.

As seen from the figure above, a separation from placebo was observed across all 12 weeks with the 133-µg/day linaclotide dose, and across all but the last week with the 266-µg/day linaclotide dose.

3.1.1.3 Reviewer's Comments and Evaluation

3.1.1.3.1 IVR Call

Proportion of patients who had completed at least 80% IVRS calls for 12 weeks of treatment for the placebo was slightly lower that for linaclotide 133 µg and 266 µg patients (72.6% vs. 75.1% and 74.6%).

Proportion of patients who had completed at least 4 IVRS calls for at least 9 of 12 weeks of treatment for the placebo and linaclotide 266 µg patients was slightly higher that for linaclotide 133 µg patients (81.9% and 81.5% vs. 77.9%).

Proportion of patients who had completed at least 4 IVRS calls for all 12 weeks of treatment for the placebo was slightly higher that for linaclotide 133 µg and 266 µg patients (69.8% vs. 63.8%and 62.9%).

3.1.1.3.2 CSBM Weekly Responder

Per request, the sponsor clarified that the determination of a patient being a 12-week CSBM Overall Responder or CSBM Weekly Responder did not incorporate the 4 compete IVRS calls criteria .However if a patient prematurely discontinued from the

trial such that the patient's final Treatment Period week contained less than 4 days, the patient was not considered a CSBM Weekly Responder for that week or the subsequent missed weeks of the Treatment Period.

The above statement is not commonly used to deal with missing daily data for CSBM weekly responder. The commonly used one is as follow:

For the primary efficacy parameter, a patient had to have ≥ 4 complete IVRS calls for a particular Treatment Period week to be considered a responder for that week.

3.1.1.3.3 Modified Intent-to-Treat Analysis of Primary Efficacy Endpoint

The sponsor performed modified ITT analysis on the primary efficacy endpoint where a subject with fewer than 4 complete IVRS calls in a Treatment Period week was considered a nonresponder for that week. The primary 12-week CSBM overall responder endpoint was then calculated based on the CSBM Weekly Responder endpoints.

The results from modified ITT analyses of 12-week CSBM overall responder are given below.

12-Week CSBM Overall Responders (Modified)
ITT Population
LIN-MD-01

Description	Placebo (N=215) n (%)	LIN 133 ug (N=213) n (%)	LIN 266 ug (N=202) n (%)
Responder	12 (5.6)	33 (15.5)	41 (20.3)
Non-responder	203 (94.4)	180 (84.5)	161 (79.7)
Difference in Responder Rate (Linaclotide - Placebo)		9.9	14.7
Odds Ratio for Response (Linaclotide : Placebo)		3.04	4.25
95% C.I. for odds ratio		(1.52, 6.06)	(2.17, 8.33)
P-value		0.0011	<.0001

Notes: A 12-week CSBM overall responder (modified) is a patient who is a CSBM weekly responder (modified) for at least 9 of the 12 weeks of the Treatment Period. A CSBM weekly responder (modified) is a patient who had a CSBM weekly frequency rate that was 3 or greater, increased by 1 or more from baseline and who completed at least 4 IVRS calls for that week.
n = Number of patients within a specific category. N = Number of patients in the ITT Population.
Odds ratio, 95% CI (Confidence Interval) and p-values were obtained from the CMH tests controlling for geographic region, comparing each linaclotide dose versus placebo in a pairwise manner.

As seen from the table above, the results were similar to those from sponsor the original analysis. Only slight changes on number of responders different from the original analysis (1, 1 and 2 less responders for placebo, linaclotide 133 µg, and linaclotide 266 µg, respectively) in the revised analysis as compared to the original analysis were observed.

3.1.1.3.4 Sensitivity Analyses of 12-Week CSBM Overall Responders

Per request, the sponsor performed the several sensitivity analyses of 12-week CSBM overall responder.

For the LOCF analysis, for any of the 12 Treatment Period weeks where a patient had less than 4 complete IVRS calls for a particular week, the patient's responder status for that week was imputed by the value of the patient's responder status from the previous Treatment Period week. If no previous Treatment Period week responder status exists, the patient was a non-responder for that week.

For this observed case analysis, if a patient had less than 4 complete IVRS calls in a Treatment Period week, that patient was considered a non-responder for that week. If a patient has less than 4 complete IVRS calls for all of the 12 weeks of the Treatment Period, that patient was excluded from the analysis.

For the complete case analysis, the primary efficacy analyses was performed on the subset of ITT Population patients who have at least 4 complete IVRS calls in each of the 12 Treatment Period weeks. Patients who had less than 4 IVRS calls for any of the 12 Treatment Period weeks were excluded from the analysis.

For the worst case analyses of the primary efficacy endpoint, two different approaches were presented, the first approach imputed a patient's overall responder status, and the second approach imputed the weekly responder status.

For the worst case imputation of overall responder status, two analyses were considered:

- Worst Case 1: If a patient had less than 4 complete calls for any of the 12 Treatment Period weeks, that patient was assumed to be "failed" and defined as a nonresponder for the trial.

- Worst Case 2: If a patient had less than 4 complete calls for any of the 12 Treatment Period weeks, that patient was a nonresponder for the trial if the patient is in one of the linaclotide treatment groups and was considered a responder for the trial if the patient is in the placebo treatment group.

For the worst case imputation of weekly responder endpoints, the following analysis will be considered:

- Worst Case 3: If a patient had less than 4 complete calls for a Treatment Period week, that patient was not a CSBM Weekly Responder for that week if the patient is in a linaclotide treatment group and was considered a CSBM Weekly Responder for that week if the patient is in the placebo treatment group. The primary overall responder endpoint was then calculated based on the CSBM Weekly Responder endpoints, using this worst case approach.

For the multiple imputation (MI) analysis, the CSBM change-from-baseline rate during a Treatment Period week was treated as missing if a patient has less than 4 complete IVRS calls during that week. All missing weekly change-from-baseline CSBM rates were imputed using MI. The MI algorithm and procedures were carried out as described in the steps below:

- The missing change-from-baseline weekly CSBM rates were imputed 20 times, resulting in multiple imputed analysis data sets.

- For each imputed dataset:

    - The primary efficacy endpoint, 12-week CSBM Overall Responder, was derived based on the imputed change-from-baseline weekly CSBM rates, following the protocol and trial SAP specified definitions for the primary efficacy endpoint

    - The primary efficacy endpoint was analyzed using the same method as specified in the trial SAPs; comparing the responder rates of each linaclotide group to placebo using a CMH test controlling for geographic region (controlling for trial and geographic region for the analysis of the two trials combined).

    - Estimates of log odds ratio (OR: linaclotide versus placebo) and its standard error was calculated.

- Then, the estimates of log (OR) and the standard error from all the imputations was be combined to obtain the overall estimates for log (OR) and its 95% confidence interval as well as the p-value for testing the null hypothesis of the log (OR) being 0. The estimated OR and 95% CI for OR was then obtained by taking the exponentiation of the point estimates for log (OR) and for the lower and upper CI limits for log (OR).

- In addition, the average responder rate and non-responder rate of all the imputed data sets for each treatment group along with the difference of the average linaclotide responder rates compared to the average placebo responder rate was also provided.

In the above MI analyses, the missing data were assumed to follow a missing at random (MAR) pattern (Little and Rubin 1987). The imputation of the change-from-baseline weekly CSBM rates was based on a multivariate normal distribution. In each imputation, a Monte-Carlo Markov chain (MCMC, see Schafer 1997) method was used to impute the missing weekly change scores. The initial mean vector and covariance matrix for the MCMC was obtained using an EM algorithm (Dempster, Laird, and Rubin 1977).

The imputed data sets were generated using PROC MI in SAS (Version 9.2) and the log odds ratios and their standard errors were combined using PROC MIANALYZE in SAS (Version 9.2).

The results from sensitivity analyses of 12-week CSBM overall responder are given below.

12-Week CSBM Overall Responders
LIN-MD-01

Analysis	PLA	LIN 133 µg	Diff	LIN 266 µg	Diff
(LOCF)	14/215 (6.5%)	47/213 (22.1%)	15.6%	51/202 (25.2%)	18.7%
Completed Case	11/150 (7.3%)	29/136 (21.3%)	14.0%	34/127 (26.8%)	19.5%
Observed Case	12/215 (5.6%)	33/209 (15.8%)	10.2%)	41/200 (20.5%	14.9%
Worst Case 1	11/215 (5.1%)	29/213 (13.6%)	8.5%	34/202 (16.8%)	11.7%
Worst Case 2	76/215 (35.3%)	29/213 (13.6%)	-11.7%	34/202 (16.8%)	-18.5
Worst Case 3	29/215 (13.5%)	33/213 (15.5%)	2.0%	41/202 (20.3%)	6.8%
Multiple Imputation	5.9%	21.1%	15.2%	26.1%	20.2%

Complied from Tables 14.4.1.1B, 14.4.1.1.D-14.4.1.1H
P- values were obtained from the CMH tests controlling for geographic region.
The complete case analysis includes only those patients who complete at least 4 IVRS calls for each of the first 12 weeks of treatment.
The observed case analysis includes only those patients who complete at least 4 IVRS calls for at least one of the first 12 weeks of treatment.
For worst case analysis 1, patients must complete at least 4 IVARS calls for each of the first 12 weeks of treatment.
For worst case analysis 2, patients who do not complete at least 4 IVRS call for each of the first 12 weeks of treatment are handled as follows: patients randomized to Linaclotide are non-responders, while patients who are randomized to placebo are considered responders.
For worst case analysis 3, for those weeks where patients do not complete at least 4 IVRS calls, patients randomized to Linaclotide are non-responders, while patients who are randomized to placebo are considered responders.

The sponsor applied the following definition for Worst Case 1:

- Worst Case 1: If a patient has less than 4 complete calls for any of the first 12 Treatment Period weeks, that patient was assumed to be “failed” and defined as a nonresponder for the trial.

Under the Worst Case 1 method, if a patient had less than 4 complete IVRS calls in any one of Treatment Period weeks 1 - 12, that patient was defined as a primary efficacy endpoint nonresponder.

In contrast, for the modified analysis for the primary efficacy endpoint in the CC trials if a patient had less than 4 complete IVRS calls for one or more of the

Treatment Period weeks 1 - 12, that patient would be defined as a weekly nonresponder for those particular weeks, but could still be a primary efficacy endpoint responder.

As such, the number of patients classified as primary efficacy endpoint responders under the Worst Case 1 method was lower than that under the modified analysis for the primary efficacy as only those patients who had at least 4 complete IVRS calls in all 12 Treatment Period weeks could potentially be primary efficacy endpoint responders under the Worst Case 1 method. As compared with the modified analysis, Worst Case 1 analysis would yield 1, 4 and 7 less responder for placebo, linaclotide 133 µg and linaclotide 266 µg, respectively..

The sponsor's Worst Case 1 analysis is one of "worst case" analyses. It was more conservative than the sponsor's modified analysis.

As seen from the table above, for the 12-week CSBM overall responder, it was shown by a significantly greater proportion of subjects taking either linaclotide 133 µg or linaclotide 266 µg compared with subjects taking placebo in all sensitivity analyses except the Worst Case 2 analysis.

Treatment difference between the linaclotide 266 µg and the linaclotide 133 µg was small; it ranged from 3.1% to 5.3%.

3.1.1.3.5 Subgroup Analyses of 12-Week CSBM Overall Responders

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 12-week CSBM overall responders for gender, age, race, region, BMI at baseline.

A summary of the results of subgroup analyses of proportion of 12- week CSBM overall responders is given in the Appendix Table 3.

As seen from Appendix Table 3, 12- week CSBM overall responder rates were reported by higher proportion of linaclotide subjects for gender, age, and, white.

3.1.1.3.6 Weekly CSBM Responder Rates by Week

As per request, the sponsor provided observed case analysis of number of subjects with weekly CSBM responder by week (see below).

Weekly CSBM Responder Rate by Treatment Group
Observed Case
Study LIN-MD-01

	PLA	LIN 133	Diff (LIN 133-PLA)	LIN 266	Diff (LIN 266 – PLA)
Week 1	22/208 (10.6%)	66/204 (32.4%)	21.8%	75/194 (38.7%)	28.1%
Week 2	33/204 (16.2%)	60/203 (29.6%)	13.4%	76/194 (39.2%)	23.0%
Week 3	23/195 (11.8%)	58/186 (31.2%)	19.4%	75/189 (39.7%)	27.9%
Week 4	32/197 (16.2%)	65/186 (34.9%)	18.7%	84/189 (44.4%)	28.2%
Week 5	23/187 (12.3%)	60/182 (33.0%)	20.7%	71/178 (39.9%)	27.6%
Week 6	25/186 (13.4%)	58/178 (32.6%)	19.1%	70/178 (39.3%)	25.9%
Week 7	31/184 (16.8%)	58/178 (32.6%)	15.7%	66/167 (39.5%)	22.7%
Week 8	30/182 (16.5%)	50/177 (28.2%)	11.8%	71/174 (40.8%)	24.3%
Week 9	26/178 (14.6%)	47/165 (28.5%)	13.9%	61/161 (37.9%)	23.3%
Week 10	23/185 (12.4%)	53/167 (31.7%)	19.3%	68/163 (41.7%)	29.3%
Week 11	23/174 (13.2%)	51/160 (31.9%)	18.7%	61/160 (38.1%)	24.9%
Week 12	30/173 (17.3%)	49/154 (31.8%)	14.5%	50/157 (31.8%)	14.5%

Compiled by this reviewer from the table 14.4.1.1k.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, greater proportions of subjects at almost every week during the course of the 12- week study in each linaclotide group compared with subjects in the placebo group was observed.

The linaclotide 266 µg was consistently numerically higher than the linaclotide 133 µg from Week 1 through Week 11. But, no treatment difference was observed at Week 12.

3.1.1.3.7 Monthly CSBM Responder

This reviewer performed analyses of CSBM monthly responder by month.

The monthly CSBM is defined that a subject be a CSBM weekly responder for at least 3 of the 4 treatment period weeks for that month. A subject with missing monthly responder at specific month was considered non-responder for that month.

The results from reviewer's analyses of monthly CSBM responder by month are given below.

Monthly CSBM Responder Rate by Treatment Group
Study LIN-MD-01
Intention-to-Treat Population

	PLA	LIN 133	Diff (LIN 133-PLA)	LIN 266	Diff (LIN 266 – PLA)
Month 1	14/215 (6.5%)	40/213 (18.8%)	12.3%	66/205 (32.2%)	25.7%
Month 2	15/215 (7.0%)	46/213 (21.6%)	14.6%	57/205 (27.8%)	20.8%
Month 3	21/215 (9.8%)	42/213 (19.7%)	9.9%	52/205 (25.4%)	15.6%

Obtained by this reviewer using the sponsor's weekly CSBM data.

As seen from the tables above, for monthly CSBM responder, greater proportions of subjects at every month during the course of the 3-month study in each linaclotide group compared with subjects in the placebo group was observed.

The linaclotide 266 µg was consistently numerically higher than the linaclotide 133 µg from Month 1 through Month 3. But, treatment difference was decreased to 5.7% at Month 3.

3.1.1.3.8 Sustained Efficacy – All 3 Months

For sustained efficacy, the commonly used endpoint for CC is the "overall responder." A subject was considered an overall responder if the subject was a month responder for all three months during 12-week study.

This "overall responder" based on monthly responders is more stringent than the pre-specified overall responder based on weekly responders.

This reviewer performed analysis of overall responder for CSBM. The results are given below.

Reviewer's "Overall Responder" Analysis by Treatment Group
Study LIN-MD-01
Intention-to-Treat Population

PLA	LIN 133	Diff (LIN 133-PLA)	LIN 266	Diff (LIN 266 – PLA)
7/215 (3.3%)	23/213 (10.8%)	7.5%	33/205 (16.1%)	12.8%

Obtained by this reviewer using the sponsor's weekly CSBM data.

As seen from the table above, for overall responder for CSBM, greater proportions of subjects in each linaclotide group compared with subjects in the placebo group was observed.

The linaclotide 266 µg was numerically higher than the linaclotide 133 µg for the overall responder. But, treatment difference of 5.3% was observed.

3.1.1.3.9 Sustained Efficacy – 9 of 12 weeks and 3 of 4 Weeks at Month 3

For sustained efficacy, the other endpoint recommended recently for CC is the "overall responder." A subject was considered an overall responder if the subject was a week responder for at least of 9 of 12 weeks and at least 3 of 4 weeks at the Month 3.

This "overall responder" based on monthly responders is more stringent than the pre-specified overall responder based on weekly responders.

This reviewer performed analysis of overall responder for CSBM. The results are given below.

Reviewer's "Overall Responder" Analysis by Treatment Group
Study LIN-MD-01
Intention-to-Treat Population

PLA	LIN 133	Diff (LIN 133-PLA)	LIN 266	Diff (LIN 266 – PLA)
12/215 (5.6%)	33/213 (15.5%)	9.9%	36/205 (17.6%)	12.0%

Obtained by this reviewer using the sponsor's weekly CSBM data.

As seen from the table above, for overall responder for CSBM, greater proportions of subjects in each linaclotide group compared with subjects in the placebo group was observed.

The linaclotide 266 μg was numerically higher than the linaclotide 133 μg for the overall responder. But, treatment difference of 2.1% was observed.

3.1.1.3.10 Reviewer's Comments on Sponsor's Controlling for Multiplicity for Primary and Secondary Efficacy Parameter

The sponsor used 5-step serial gatekeeping multiple comparison procedure to control type 1 family-wise error rate for testing the primary and secondary efficacy parameters.

The detailed procedure is listed below.

The overall type I family-wise error rate for testing the primary and secondary efficacy parameters was controlled at the 0.05 significance level using the following 5-step serial gatekeeping multiple comparisons procedure (MCP). Following this MCP, progression to the next step only occurred if all individual hypotheses within a step were rejected and the previous step(s) were all rejected at the step-specific overall significance level. If all hypotheses within a step were not rejected, the hypothesis tests involved in all subsequent steps were considered not statistically significant. All hypothesis tests were 2-sided.

1. The first step tested the primary efficacy parameter for the 266 µg group at the 0.05 significance level
2. The second step tested the primary efficacy parameter for the 133 µg group and the first 5 secondary parameters (i.e., CSBM Frequency, SBM frequency, Stool Consistency, Severity of Straining, and Constipation Severity) for the 266 µg group. The 6 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters.
3. The third step tested the last 2 secondary efficacy parameters (i.e., Bloating and Abdominal Discomfort) for the 266 µg group. The 2 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters.
4. The fourth step tested the first 5 secondary efficacy parameters for the 133 µg group. The 5 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters.
5. The fifth step tested the last 2 secondary efficacy parameters for the 133 µg group. The 2 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters.

This reviewer's comments on this gatekeeping procedure were

> The sponsor's gatekeeping procedure was not appropriate. The Hochberg procedure is generally not recommended for sequential testing. It is not assumption free. Furthermore, it is known to provide overall α-control for independent and for certain types of positive correlated endpoints. But its properties for other types of dependent endpoints are not fully known. Various simulation experiments indicate that this method generally controls the overall Type 1 error rate for positive correlated endpoints but fails to do so for some negatively correlated endpoints.
>
> The sponsor should use a Bonferroni based gatekeeping procedure to test all endpoints in the primary family and proceed to the secondary family of endpoints only if there has been statistical success in the primary family.

Furthermore, since p-values for most secondary endpoints were very small (<0.001), all secondary endpoints would pass any statistical procedure for controlling the type 1 error for multiplicity.

3.1.1.3.11 Reviewer's Comments on Results of Secondary Efficacy Endpoints

The sponsor's pre-specified analysis for the secondary endpoints was based on a modeling approach (ANCOVA) using all data for each week 1-12. The term "treatment overall" refers to an average treatment effect over the 12 weeks of the study. (b) (4)

The sponsor's the summary of secondary efficacy endpoints is given below.

Summary of Secondary Efficacy Endpoints
Study LIN-MD-01
(ITT Population)

Parameter	*Mean Baseline*	*Placebo (N = 215) LS Mean Change (SE)*	*Linaclotide*			
			145 ug (N = 213)		*290 ug (N = 202)*	
			LS Mean Change (SE)	**LSMD (95% CI)**	**LS Mean Change (SE)**	**LSMD (95% CI)**
CSBMs/Week	0.3	0.6 (0.2)	2.0[b] (0.2)	1.4 (0.9, 1.9)	2.7[b] (0.2)	2.0 (1.5, 2.6)
SBMs/Week	1.9	1.1 (0.3)	3.4[b] (0.3)	2.3 (1.7, 3.0)	3.7[b] (0.3)	2.6 (1.9, 3.2)
Stool Consistency (BSFS Score)	2.3	0.6 (0.1)	1.8[b] (0.1)	1.3 (1.0, 1.5)	2.0[b] (0.1)	1.4 (1.2, 1.7)
Severity of Straining (5-point Ordinal Scale)	3.3	-0.6 (0.1)	-1.1[b] (0.1)	-0.6 (-0.7, -0.4)	-1.2[b] (0.1)	-0.7 (-0.8, -0.5)
Abdominal Discomfort (5-point Ordinal Scale)	2.5	-0.3 (0.0)	-0.5[a] (0.0)	-0.2 (-0.3, -0.1)	-0.5[b] (0.0)	-0.2 (-0.3, -0.1)
Bloating (5-point Ordinal Scale)	2.8	-0.2 (0.0)	-0.4[a] (0.0)	-0.2 (-0.3, -0.1)	-0.5[b] (0.0)	-0.3 (-0.4, -0.1)
Constipation Severity (5-point Ordinal Scale)	3.3	-0.3 (0.1)	-0.9[b] (0.1)	-0.6 (-0.8, -0.5)	-1.0[b] (0.1)	-0.6 (-0.8, -0.5)

The mean change from baseline is a least-squares mean change based on an ANCOVA model with treatment group and geographic region as factors and baseline value as covariate.

Baseline is the mean value for the combined ITT Population.

CI = confidence interval; LS = least squares; LSMD = least squares mean difference; SE = standard error of LS mean.

p-values based on a pairwise comparison versus placebo in an ANCOVA model.

a. $p < 0.001$

b. $p < 0.0001$

Source: LIN-MD-01 Tables 14.2.4 and 14.4.2.1A to 14.4.2.7A

As seen from the table above, secondary efficacy endpoints were statistically significantly improved for both doses of linaclotide compared with placebo. However, treatment differences for changes from baseline for severity of straining, abdominal discomfort, bloating and constipation severity might not be clinically meaningful.

Additionally, for the changes from baseline for CSBMs/week and SBMs/week, the treatment effect was slightly numerically greater for subjects in the 290 µg dose group than for those in the 145 µg dose group. But, these treatment differences of 0.7 and 0.3 might not be clinical meaningful.

Per request, the sponsor also performed sensitivity analyses for secondary efficacy endpoints. The results are summarized in the Appendix Table 4.

As seen from Appendix Table 4, all sensitivity analyses (LOCF, CC, OC, BOCF and MI) gave similar results.

3.1.2 Study MCP-103-303

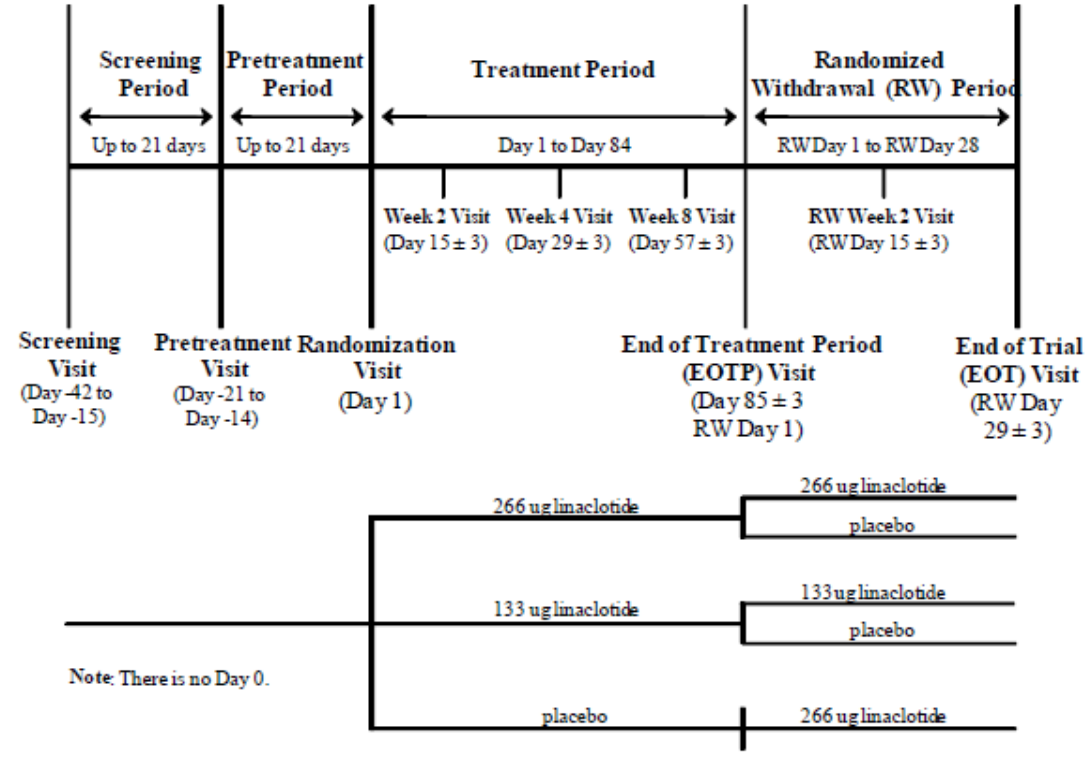
3.1.2.1 Study Design

This study was a phase III, randomized, double-blind, placebo-controlled, parallel-group trial of 133 and 266 µg linaclotide administered orally for 12 Weeks followed by a 4-Week randomized withdrawal period in patients with patients with CC. The trial was conducted in the U.S in 110 sites.

The study design of this study was similar to that of Study LIN-MD-01 with exception listed below.

This trial consisted 4 distinct periods (Figure 1).

Figure 1. Illustration of Trial Design



As shown in the figure below, the trial consisted of up to 21 days of screening (screening period), 14 to 21 days of pretreatment (pretreatment period), 12 weeks of double-blind treatment (treatment period), and a 4-week double-blind randomized withdrawal (RW) period.

The RW Period was defined as the 4 weeks immediately following the Treatment Period. The beginning of the RW Period coincided with the end of the Treatment Period. Patients who completed the 12-week Treatment Period entered the 4-week double-blind RW Period and, in a double-blind manner, were allocated to study drug as follows:

- Patients randomized to 266 µg linaclotide during the Treatment Period were re-randomized to 266 µg linaclotide or placebo (1:1).
- Patients randomized to 133 µg linaclotide during the Treatment Period were re-randomized to 133 µg linaclotide or placebo (1:1).
- Patients randomized to placebo during the Treatment Period were allocated to 266 µg linaclotide.

Study drug was taken once daily in the morning ≥ 30 minutes before breakfast. Patients continued to call the IVRS to provide their daily assessments, weekly assessments, and rescue medication use. A Treatment Satisfaction Assessment was performed at all RW Period visits. At the EOT Visit, patients completed the Treatment Continuation Assessment and some of the quality of life and patient-outcome assessments.

For the RW Period, descriptive statistics and confidence intervals are presented by Treatment Sequence for the following parameters: change from baseline in CSBM weekly frequency, change from baseline in SBM weekly frequency, change from baseline in stool consistency, change from baseline in severity of straining, change from baseline in abdominal discomfort, change from baseline in bloating, change from baseline in constipation severity, and change from baseline in percentage of days of using per-protocol rescue medication or any other laxative, suppository, or enema. Weekly summaries of these parameters are presented by Treatment Sequence for the 16-week Treatment-RW Period. For RW Week 2 and EOT Visits, treatment satisfaction is summarized (descriptive statistics) for each of 5 Treatment Sequences. For the EOT Visit, treatment continuation is summarized (descriptive statistics) for each of the 5 Treatment Sequences.

3.1.2.2 Sponsor's Analysis

A total of 1147 patients were screened. Two hundred-five patients were screen failures and 299 patients were pretreatment failures. Six hundred forty-three (643) patients provided informed consent, successfully completed Screening and the Pretreatment Period, and were randomized to treatment. Five hundred-forty (84%) of the 643 randomized patients completed the Treatment Period per protocol requirements. A total of 103 patients withdrew from the trial during the 12-week Treatment Period.

The disposition of the patients in the treatment period was summarized below.

Number (%) of Patients Discontinued During Treatment Period
Randomized Population
Study MCP-103-303

	Placebo (N=209) n (%)	Lin 133 ug (N=217) n (%)	Lin 266 ug (N=217) n (%)	Total (N=643) n (%)
Completed Treament Period [1]	177 (84.7)	186 (85.7)	177 (81.6)	540 (84.0)
Prematurely Discontinued	32 (15.3)	31 (14.3)	40 (18.4)	103 (16.0)
P-value		0.7863	0.4385	
Reason for Premature Discontinuation				
Adverse Event	8 (3.8)	11 (5.1)	10 (4.6)	29 (4.5)
P-value		0.6411	0.8111	
Protocol Violation	4 (1.9)	2 (0.9)	6 (2.8)	12 (1.9)
P-value		0.4419	0.7514	
Withdrawal of Consent	8 (3.8)	12 (5.5)	12 (5.5)	32 (5.0)
P-value		0.4946	0.4946	
Lost to Follow-up	3 (1.4)	4 (1.8)	10 (4.6)	17 (2.6)
P-value		1.0000	0.0881	
Insufficient Therapeutic Response	8 (3.8)	1 (0.5)	2 (0.9)	11 (1.7)
P-value		0.0183	0.0582	
Study Terminated by Sponsor	0	0	0	0
Other Reasons	1 (0.5)	1 (0.5)	0	2 (0.3)
P-value		1.0000	0.4906	

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A total of 12 randomized patients had deviations from the study inclusion/exclusion criteria. The reasons for these protocol deviations included: 1 patient taking prohibited medications; 8 patients not meeting colonoscopy requirements; 1 patient with fecal impaction requiring hospitalization or emergency room treatment, or history of cathartic colon, laxative or enema abuse, ischemic colitis, or pelvic floor dysfunction; 1 patient with urine pregnancy test not performed at Screening (Inclusion Criterion 4); and 1 patient with a segment of GI tract removed.

Two patients received the incorrect dose in the trial. Patient 0273002 was randomized to 133 µg, but at Visit 6, was dispensed 266 µg due to human error in dispensing the correct kit. The patient remained on the incorrect dose from 27 March 2009 through 23 April 2009, after which she was re-randomized to 133 µg linaclotide in the RW Period. Patient 0393006 received the incorrect dose for the duration of the RW Period (133 µg instead of placebo). Both patients were analyzed as randomized.

The Randomized Population included 643 patients who were randomized to a treatment group at the Randomization Visit.

The Safety Population included 643 patients who received ≥ 1 dose of double-blind study drug during the Treatment Period.

The ITT Population included 642 patients who were in the Safety Population and had ≥ 1 post-randomization entry of the primary efficacy assessment.

The RW Analysis Population included 538 patients who were re-randomized into the RW Period and had ≥ 1 dose of double-blind study drug during the RW Period. Patients were analyzed using 5 Treatment Sequences.

3.1.2.2.1 Planned Analysis

Planned analysis for this study was similar to that for Study LIN-MD-01.

3.1.2.2.2 Treatment Group Comparability

A summary of the results of the comparability of treatment groups at baseline for all randomized patients is given in the Appendix Tables 5 and 6.

As seen from Appendix Table 5, in the ITT population, the median age of subjects was 48 years. Most subjects were white (75%), and the majority were female (87%). The treatment groups were generally balanced with respect to baseline demographics and baseline characteristics except for age and Hispanic/Latino ethnicity. Mean patient age for all patients was 48.0 years; means for individual dose groups were 49.3 years for placebo, 47.1 years for the 133 µg linaclotide group, and 47.6 years for the 266 µg linaclotide group. More patients reported Hispanic/Latino ethnicity in the 266 µg group (15 patients, 6.9%) compared to placebo (6 patients, 2.9%) (p= 0.0399).

As seen from Appendix Table 6, overall, baseline efficacy parameters were similar for each active treatment group compared to placebo.

Overall for the Treatment Period, 191 (91.4%) of 209 placebo patients and 391 (90.1%) of 434 linaclotide patients received at least 1 concomitant medication during the trial.

Overall, treatment compliance was over 96% for all dosing groups during the Treatment Period (placebo = 96.7%, linaclotide 133 µg = 96.5%, and linaclotide 266 µg = 96.9%). The compliance rate remained steady and above the 96% level for all groups throughout Weeks 1-4, Weeks 5-8, and Weeks 9-12.

Overall, the percentage of patients who were ≥ 80% IVRS compliant during the 2-week Pretreatment Period was 97% for placebo, 97% for linaclotide, 133 µg and 95% for linaclotide 266 µg. During the 12-week Treatment Period, 84%, 80%, and 78% of placebo, 133 µg, and 266 µg patients had a complete IVRS call at least 80% of the time.

3.1.2.2.3 Sponsor's Analysis of Primary Efficacy Variable

The primary efficacy endpoint was the number of patients who were 12-week CSBM overall responders, defined as patients who were CSBM responders for at least 9 of the 12 weeks of the treatment period; a CSBM weekly responder was a patient who

had a CSBM weekly frequency rate that was 3 or greater and increased by 1 or more from baseline.

The result from analysis of 12-week CSBM overall responders in the ITT population is given below.

Primary Efficacy Analyses: 12-Week CSBM Overall Responders
ITT Population
MCP-103-303

		Linaclotide	
Description	Placebo (N=209) n (%)	133 ug (N=217) n (%)	266 ug (N=216) n (%)
Responder	7 (3.3)	46 (21.2)	42 (19.4)
Non-Responder	202 (96.7)	171 (78.8)	174 (80.6)
Difference in Responder Rate (Linaclotide - Placebo)		17.8	16.1
Odds Ratio for Response (Linaclotide : Placebo)		7.72	7.21
95% CI for Odds Ratio		(3.41, 17.47)	(3.14, 16.59)
P-value		< 0.0001	< 0.0001

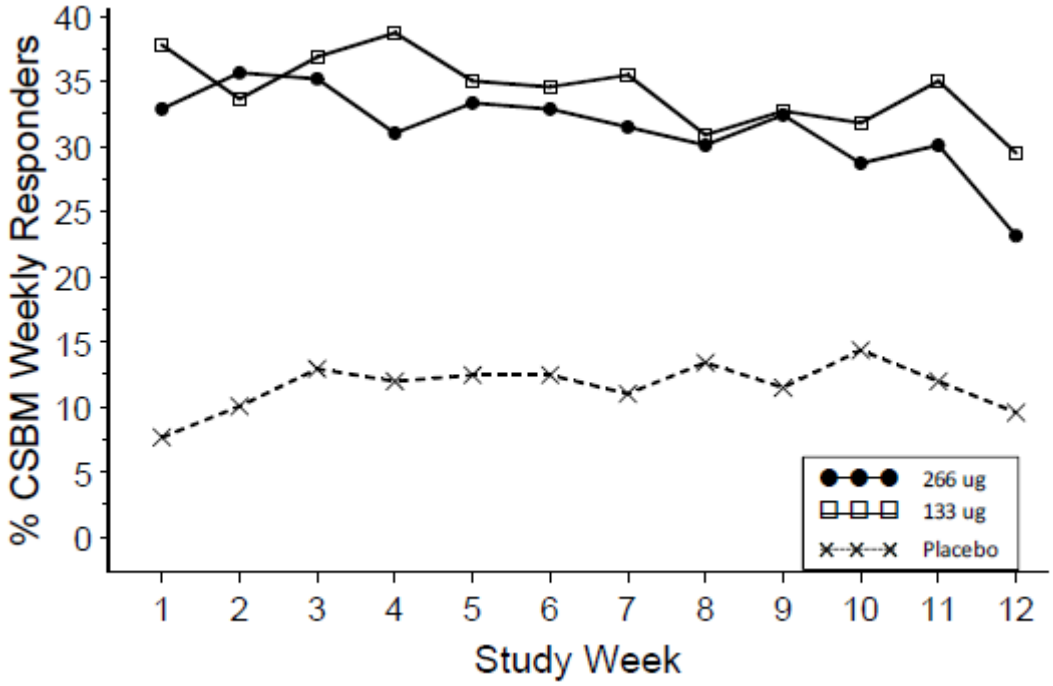
Data Source: Section 14, Table 14.4.1.1
A 12-week CSBM Overall Responder is a patient who was a CSBM Weekly Responder for at least 9 of the 12 weeks of the Treatment Period. A CSBM Weekly Responder is a patient who had a CSBM weekly frequency rate that was 3 or greater and increased by 1 or more from baseline
n = Number of patients within a specific category.
CI = Confidence interval
Odds ratios were estimated using the Mantel-Haenszel method controlling for geographic region.
P-values were obtained from the CMH tests controlling for geographic region, comparing each linaclotide dose versus placebo in a pairwise manner.
Both p-values met the criterion for statistical significance based on the MCP.

As seen from the table above, the number and percentage of patients who were 12-Week CSBM overall responders were greater for each linaclotide group when compared to placebo.

3.1.2.2.4 CSBM Weekly Responders and Improvement in 12-Week CSBM Rate at Incremental Levels

The sponsor also performed analysis of CSBM weekly responders by week. The percentage of patients who were CSBM weekly responders are presented graphically below as supportive to the primary efficacy parameter (Section 3.1.2.2.3.) Discontinued patients were considered CSBM nonresponders for those weeks subsequent to their discontinuation.

Percent CSBM Weekly Responders—ITT Population
MCP-103-303



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As seen from the figure above, during each week of the treatment period, the proportion of patients who were CSBM weekly responders (patients who had ≥ 3 CSBMs and a change from baseline of ≥1 during the particular week) was greater with each dose of linaclotide than with placebo.

3.1.2.2.5 Sponsor's Analyses of Secondary Variables

The secondary efficacy parameters based on the IVRS calls were:

- Change from baseline in 12-week CSBM frequency rate
- Change from baseline in 12-week SBM frequency rate
- Change from baseline in 12-week stool consistency
- Change from baseline in 12-week severity of straining
- Change from baseline in 12-week abdominal discomfort
- Change from baseline in 12-week bloating
- Change from baseline in 12-week constipation severity

3.1.2.2.5.1Change from Baseline in 12-week CSBM Frequency Rate

A summary of the results of the analysis of the change from baseline in 12-week CSBM frequency rate (i.e., weekly CSBM frequency rate over the 12-week treatment) is given below.

Change From Baseline in 12-Week CSBM Frequency Rate
ITT Population
MCP-103-303

Visit	Statistic	Placebo (N=209)	Linaclotide 133 ug (N=217)	Linaclotide 266 ug (N=216)
Baseline	Mean	0.333	0.332	0.239
	SD	0.591	0.569	0.448
	SEM	0.041	0.039	0.031
	Median	0.000	0.000	0.000
	Min, Max	0.00, 2.43	0.00, 2.90	0.00, 1.95
	n	209	217	216
Treatment Overall	Mean	0.911	2.384	2.389
	SD	1.380	2.549	2.929
	SEM	0.095	0.173	0.199
	Median	0.320	1.659	1.541
	Min, Max	0.00, 10.17	0.00, 11.90	0.00, 18.74
	n	209	217	216
ANCOVA Results	LS Mean Change from Baseline (SE)	0.453 (0.169)	1.935 (0.167)	2.042 (0.167)
	LS Mean Difference (95% CI) [Linaclotide - Placebo]		1.482 (1.04, 1.92)	1.589 (1.15, 2.03)
	P-value[a]		< 0.0001	< 0.0001

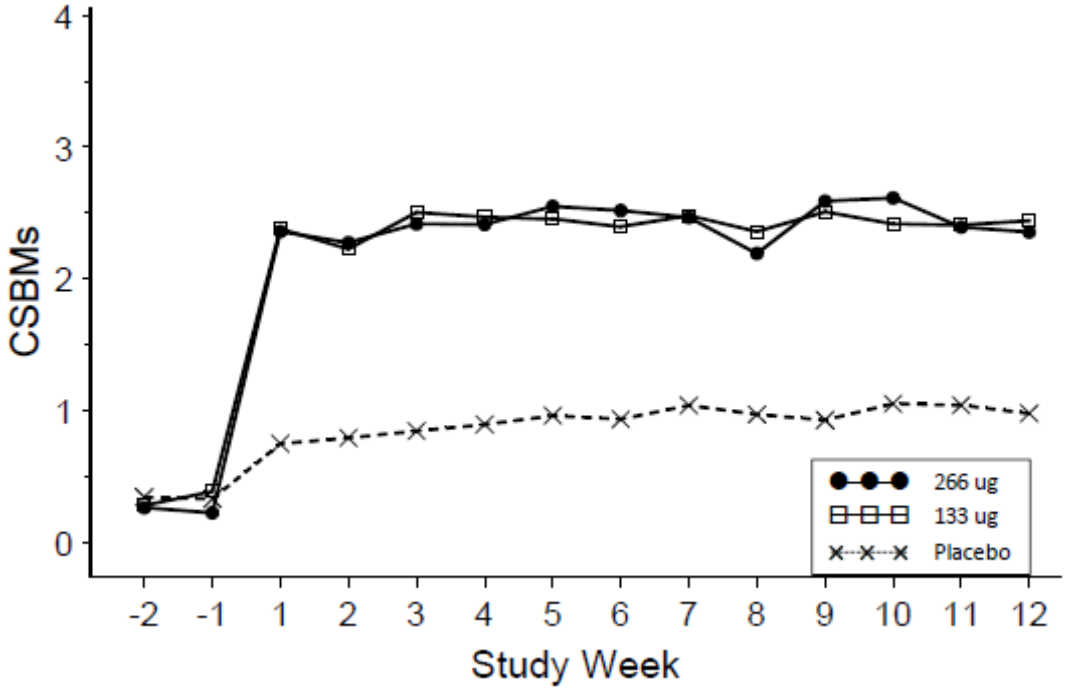
Data source: Section 14, Table 14.4.2.1A
A patient's 12-week CSBM Frequency Rate is the CSBM rate (CSBMs/week) calculated over the 12-weeks of the Treatment Period.
n = Number of patients with analysis values at both baseline and a specific time point in the ITT Population.
SD = standard deviation, SEM = standard error of the mean, Min = minimum, Max = maximum and
SE = Standard Error of LS Mean.
[a] P-values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. P-values are less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the mean baseline values for CSBM frequency rates were low for all three groups, indicating a highly constipated patient population overall; however, the linaclotide 266 µg group had slightly lower baseline rates when compared to both placebo and linaclotide 133 µg.

The LS mean change from baseline in CSBM frequency for the linaclotide 133 µg and 266 µg groups were numerically greater than placebo, and the difference was statistically significant for both linaclotide doses compared to placebo.

Mean CSBM frequency rates during the treatment period are plotted by week and is given below.

Mean CSBM Rate (OC) by Week (Treatment Period)
ITT Population
MCP-103-303



Copied from Figure 6.

As seen from the figure above, linaclotide treatment separated from placebo treatment during Week 1 and was sustained across the 12-week treatment period.

3.1.1.2.5.2 Change from Baseline in 12-week SBM Frequency Rate

A summary of the results of the analysis of the change from baseline in 12-week SBM frequency rate (i.e., weekly SBM frequency rate over the 12-week treatment) is given below.

Change From Baseline in 12-Week SBM Frequency Rate
ITT Population
MCP-103-303

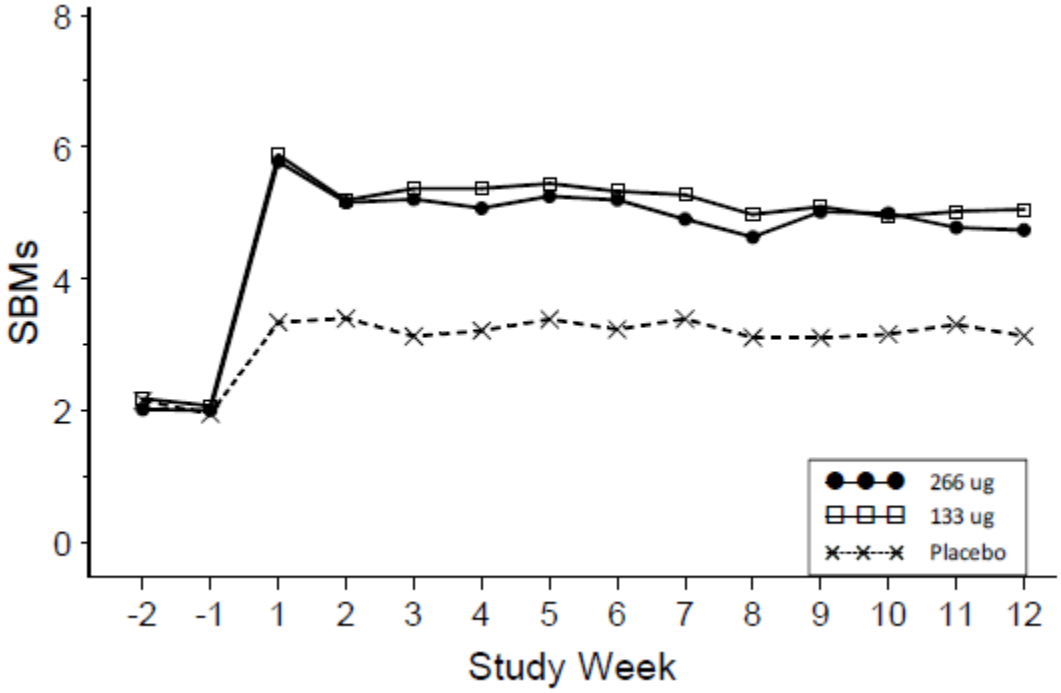
Visit	Statistic	Placebo (N=209)	Linaclotide 133 ug (N=217)	Linaclotide 266 ug (N=216)
Baseline	Mean	2.047	2.126	2.011
	SD	1.628	1.629	1.634
	SEM	0.113	0.111	0.111
	Median	1.926	1.937	1.465
	Min, Max	0.00, 6.80	0.00, 6.78	0.00, 7.33
	n	209	217	216
Treatment Overall	Mean	3.214	5.237	5.089
	SD	2.293	3.302	4.058
	SEM	0.159	0.224	0.276
	Median	3.223	4.978	4.343
	Min, Max	0.00, 15.09	0.00, 15.70	0.00, 19.07
	n	209	217	216
ANCOVA Results	LS Mean Change from Baseline (SE)	1.075 (0.216)	3.034 (0.213)	2.982 (0.213)
	LS Mean Difference (95% CI) [Linaclotide - Placebo]		1.959 (1.40, 2.52)	1.907 (1.35, 2.47)
	P-value[a]		< 0.0001	< 0.0001

Data source: Section 14, Table 14.4.2.2A
A patient's 12-week SBM Frequency Rate is the SBM rate (SBMs/week) calculated over the 12-weeks of the Treatment Period.
n = Number of patients with analysis values at both baseline and a specific time point in the ITT Population.
SD=standard deviation, SEM=standard error of the mean, Min=minimum, Max=maximum and SE = Standard Error of LS Mean.
[a] P-values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. P-values are less than the threshold value for statistical significance based on the MCP

As seen from the table above, the LS mean change from baseline in SBM frequency for the linaclotide 133 µg and 266 µg groups were numerically greater than placebo, and the difference was statistically significant for both linaclotide doses compared to placebo.

Mean SBM frequency rate during the treatment period is graphed below by week.

Mean SBM Rate (OC) by Week (Treatment Period)
ITT Population
MCP-103-303



Copied from Figure 7.

As seem from the figure above, both the 133- and 266-µg/day doses demonstrated a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.2.2.5.3 Change from Baseline in 12-week Stool Consistency

A summary of the results of the analysis of the change from baseline in 12-week stool consistency is given below.

Change From Baseline in 12-Week Stool Consistency
ITT Population
MCP-103-303

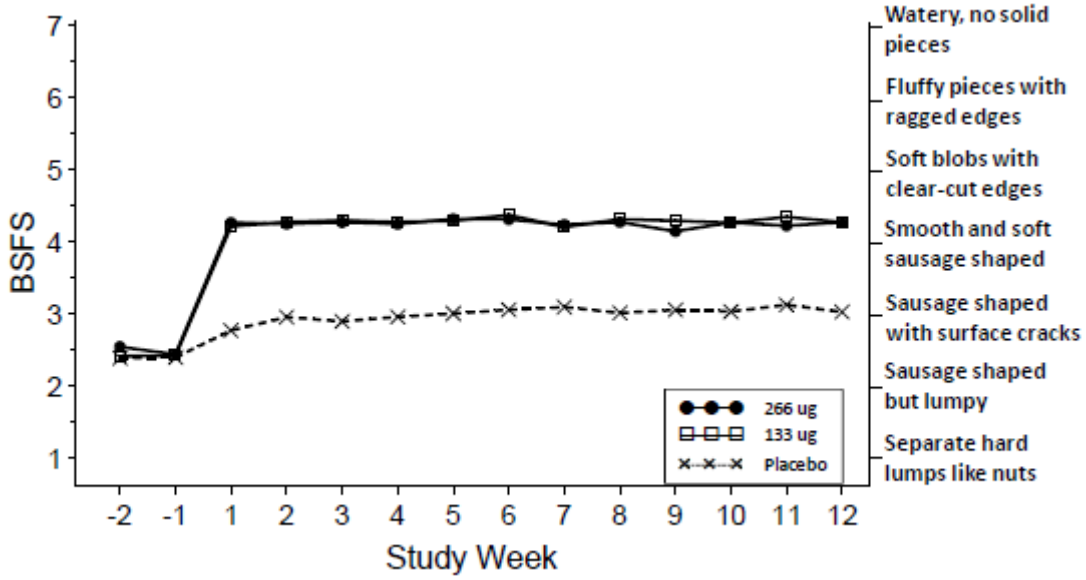
Visit	Statistic	Placebo (N=209)	Linaclotide 133 ug (N=217)	Linaclotide 266 ug (N=216)
Baseline	Mean	2.382	2.378	2.516
	SD	1.000	0.974	1.058
	SEM	0.075	0.072	0.079
	Median	2.250	2.333	2.500
	Min, Max	1.00, 6.00	1.00, 6.00	1.00, 6.00
	n	177	183	181
Treatment Overall	Mean	3.004	4.285	4.323
	SD	0.958	1.219	1.173
	SEM	0.072	0.090	0.087
	Median	2.885	4.368	4.351
	Min, Max	1.00, 6.53	1.17, 6.79	1.33, 6.74
	n	177	183	181
ANCOVA Results	LS Mean Change from Baseline (SE)	0.576 (0.085)	1.851 (0.084)	1.838 (0.084)
	LS Mean Difference (95% CI) [Linaclotide - Placebo]		1.275 (1.06, 1.49)	1.263 (1.04, 1.48)
	P-value[a]		< 0.0001	< 0.0001

Data source: Section 14, Table 14.4.2.3A
Stool consistency was measured daily using the seven-point ordinal BSFS (1 = separate hard lumps like nuts (difficult to pass); 2 = sausage shaped but lumpy; 3 = like a sausage but with cracks on surface; 4 = like a sausage or snake, smooth and soft; 5 = soft blobs with clear-cut edges (passed easily); 6 = fluffy pieces with ragged edges, a mushy stool; 7 = watery, no solid pieces (entirely liquid)).
The patient's BSFS score for the Treatment Period is the average of the nonmissing BSFS scores from the SBMs reported by the patient during the 12-week Treatment Period.
n = Number of patients with analysis values at both baseline and a specific time point in the ITT Population.
SD=standard deviation, SEM=standard error of the mean, Min=minimum, Max=maximum and SE = Standard Error of LS Mean.
[a] P-values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. P-values are less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the LS mean change from baseline in stool consistency for the linaclotide 133 µg and 266 µg groups were numerically greater than placebo, and the difference was statistically significant for both linaclotide doses compared to placebo.

Mean stool consistency during the treatment period is graphed below by week.

Mean Stool Consistency (OC) by Week (Treatment Period)
ITT Population
MCP-103-303



Copied from Figure 8.

As seem from the figure above, both the 133- and 266-µg/day doses demonstrated a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.2.2.5.4 Change from Baseline in 12-week Severity of Straining

A summary of the results of the analysis of the change from baseline in 12-week severity of straining is given below.

Change From Baseline in 12-Week Severity of Straining
ITT Population
MCP-103-303

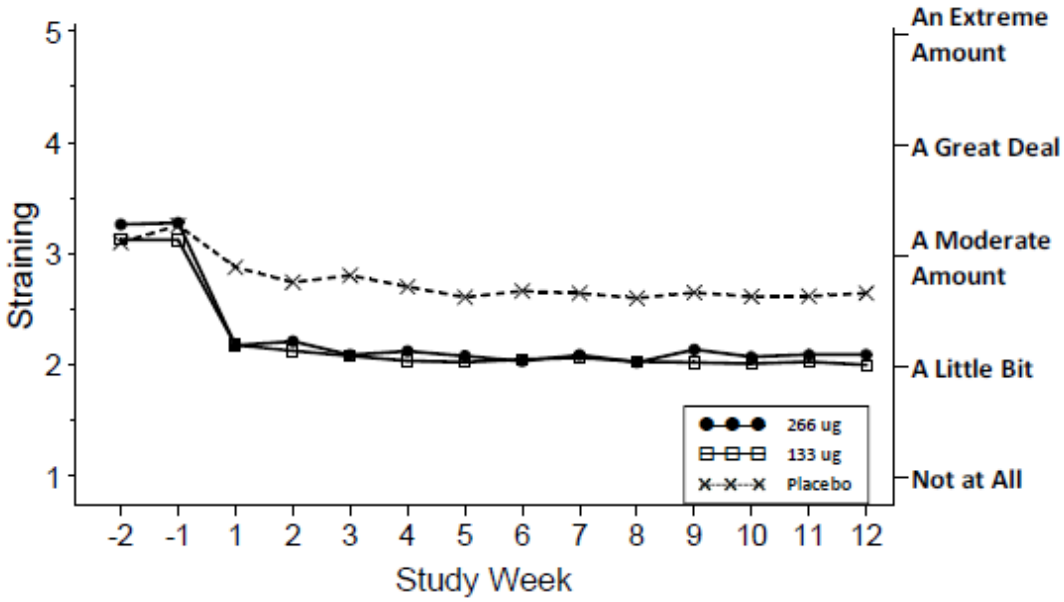
Visit	Statistic	Placebo (N=209)	Linaclotide 133 ug (N=217)	Linaclotide 266 ug (N=216)
Baseline	Mean	3.178	3.171	3.293
	SD	0.887	0.811	0.872
	SEM	0.067	0.060	0.065
	Median	3.200	3.000	3.250
	Min, Max	1.00, 5.00	1.00, 5.00	1.00, 5.00
	n	177	183	181
Treatment Overall	Mean	2.667	2.059	2.061
	SD	0.692	0.664	0.699
	SEM	0.052	0.049	0.052
	Median	2.644	2.000	1.978
	Min, Max	1.06, 4.43	1.00, 4.21	1.00, 4.33
	n	177	183	181
ANCOVA Results	LS Mean Change from Baseline (SE)	-0.512 (0.050)	-1.119 (0.050)	-1.150 (0.050)
	LS Mean Difference (95% CI) [Linaclotide - Placebo]		-0.606 (-0.74, -0.48)	-0.637 (-0.77, -0.51)
	P-value[a]		< 0.0001	< 0.0001

Data source: Section 14, Table 14.4.2.4A
Severity of Straining was measured daily using a five-point ordinal scale (1 = not at all; 2 = a little bit; 3 = a moderate amount; 4 = a great deal; 5 = an extreme amount). The patient's straining score for the Treatment Period is the average of the nonmissing straining scores from the SBMs reported by the patient during the 12-week Treatment Period.
n = Number of patients with analysis values at both baseline and a specific time point in the ITT Population.
SD=standard deviation, SEM=standard error of the mean, Min=minimum, Max=maximum and SE = Standard Error of LS Mean.
[a] P-values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. P-values are less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the LS mean change from baseline in severity of straining for the linaclotide 133 µg and 266 µg groups were numerically greater than placebo, and the difference was statistically significant for both linaclotide doses compared to placebo.

Mean severity of straining during the treatment period is graphed below by week.

Mean Severity of Straining (OC) by Week (Treatment Period)
ITT Population
MCP-103-303



Copied from Figure 9.

As seem from the figure above, both the 133- and 266-µg/day doses demonstrated a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.2.2.5.5 Change from Baseline in 12-week Abdominal Discomfort

A summary of the results of the analysis of the change from baseline in 12-week abdominal discomfort is given below.

Change From Baseline in 12-Week Abdominal Discomfort
ITT Population
MCP-103-303

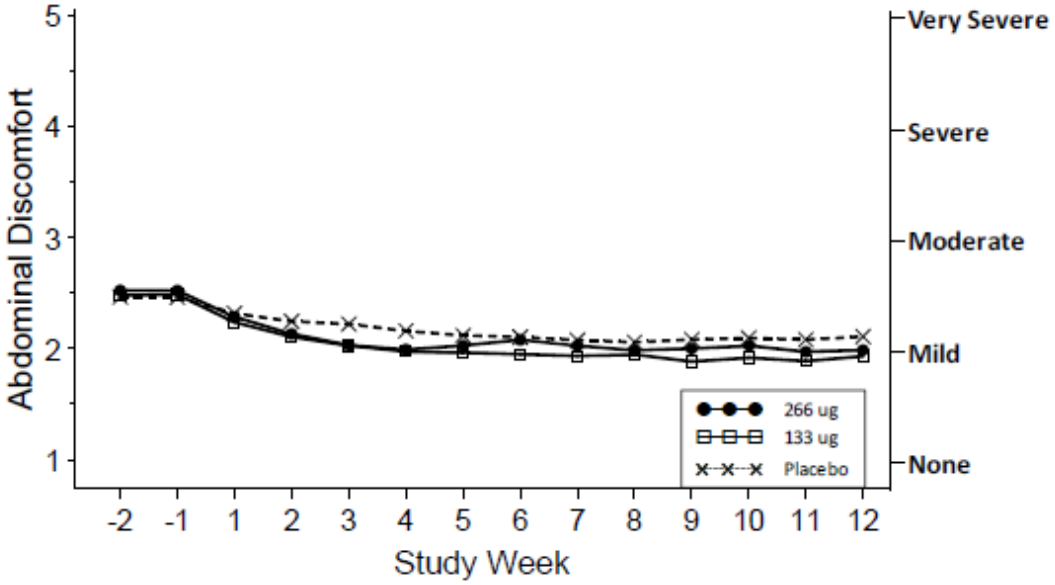
Visit	Statistic	Placebo (N=209)	Linaclotide 133 ug (N=217)	Linaclotide 266 ug (N=216)
Baseline	Mean	2.464	2.482	2.522
	SD	0.814	0.808	0.832
	SEM	0.056	0.055	0.057
	Median	2.538	2.467	2.586
	Min, Max	1.00, 4.29	1.00, 4.87	1.00, 4.71
	n	209	217	216
Treatment Overall	Mean	2.155	1.994	2.060
	SD	0.720	0.713	0.712
	SEM	0.050	0.048	0.048
	Median	2.100	1.916	1.988
	Min, Max	1.00, 4.72	1.00, 4.77	1.00, 3.98
	n	209	217	216
ANCOVA Results	LS Mean Change from Baseline (SE)	-0.303 (0.037)	-0.478 (0.036)	-0.435 (0.036)
	LS Mean Difference (95% CI) [Linaclotide - Placebo]		-0.175 (-0.27, -0.08)	-0.133 (-0.23, -0.04)
	P-value[a]		0.0003	0.0063

Data source: Section 14, Table 14.4.2.6A
Abdominal Discomfort was measured daily using a five-point ordinal scale (1 = none; 2 = mild; 3 = moderate; 4 = severe; 5 = very severe). The patient's Abdominal Discomfort score for the Treatment Period is the average of the nonmissing daily patient assessments of Abdominal Discomfort scores reported during the 12-week Treatment Period.
n = Number of patients with analysis values at both baseline and a specific time point in the ITT Population.
SD=standard deviation, SEM=standard error of the mean, Min=minimum, Max=maximum and SE = Standard Error of LS Mean.
[a] P-values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. P-values are less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the LS mean change from baseline in abdominal discomfort for the linaclotide133 µg and 266 µg groups were slightly numerically greater than placebo, and the difference was statistically significant for both linaclotide doses compared to placebo.

Mean abdominal discomfort during the treatment period is graphed below by week.

Mean Abdominal Discomfort (OC) by Week (Treatment Period)
ITT Population
MCP-103-303



Copied from Figure 10.

As seem from the figure above, both the 133- and 266-µg/day doses failed to demonstrate a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.1.2.5.6 Change from Baseline in 12-week Bloating

A summary of the results of the analysis of the change from baseline in 12-week bloating is given below.

Change From Baseline in 12-Week Bloating
ITT Population
MCP-103-303

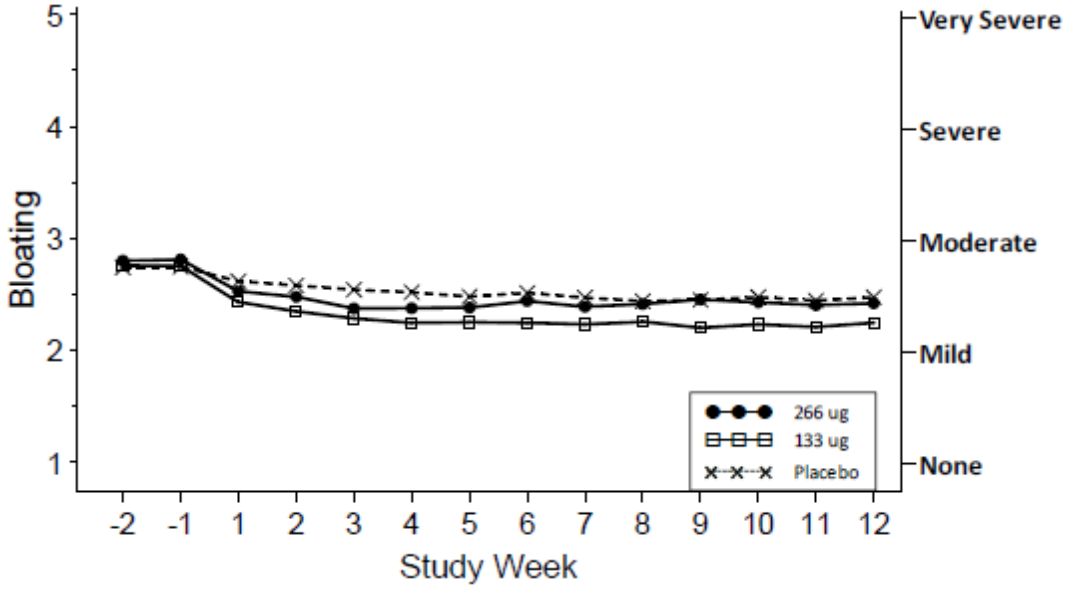
Visit	Statistic	Placebo (N=209)	Linaclotide 133 ug (N=217)	Linaclotide 266 ug (N=216)
Baseline	Mean	2.742	2.755	2.805
	SD	0.867	0.870	0.869
	SEM	0.060	0.059	0.059
	Median	2.800	2.800	2.800
	Min, Max	1.00, 4.79	1.00, 5.00	1.00, 5.00
	n	209	217	216
Treatment Overall	Mean	2.512	2.283	2.408
	SD	0.815	0.815	0.834
	SEM	0.056	0.055	0.057
	Median	2.568	2.275	2.345
	Min, Max	1.00, 4.73	1.00, 5.00	1.00, 4.73
	n	209	217	216
ANCOVA Results	LS Mean Change from Baseline (SE)	-0.223 (0.040)	-0.464 (0.040)	-0.373 (0.040)
	LS Mean Difference (95% CI) [Linaclotide - Placebo]		-0.240 (-0.34, -0.14)	-0.150 (-0.25, -0.05)
	P-value[a]		< 0.0001	0.0049

Data source: Section 14, Table 14.4.2.7A
Bloating was measured daily using a five-point ordinal scale (1 = none; 2 = mild; 3 = moderate; 4 = severe; 5 = very severe). The patient's Bloating score for the Treatment Period is the average of the nonmissing daily patient assessments of Bloating scores reported during the 12-week Treatment Period.
n = Number of patients with analysis values at both baseline and a specific time point in the ITT Population.
SD=standard deviation, SEM=standard error of the mean, Min=minimum, Max=maximum and SE = Standard Error of LS Mean.
[a] P-values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. P-values are less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the LS mean change from baseline in abdominal discomfort for the linaclotide 133 µg and 266 µg groups were slightly numerically greater than placebo, and the difference was statistically significant for both linaclotide doses compared to placebo.

Mean bloating during the treatment period is graphed below by week.

Mean Bloating (OC) by Week (Treatment Period)
ITT Population
MCP-103-303



Copied from Figure 11.

As seem from the figure above, both the 133- and 266-µg/day doses failed to demonstrate a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.1.2.5.7 Change from Baseline in 12-week Constipation Severity

A summary of the results of the analysis of the change from baseline in 12-week constipation severity is given below.

Change From Baseline in 12-Week Constipation Severity
ITT Population
MCP-103-303

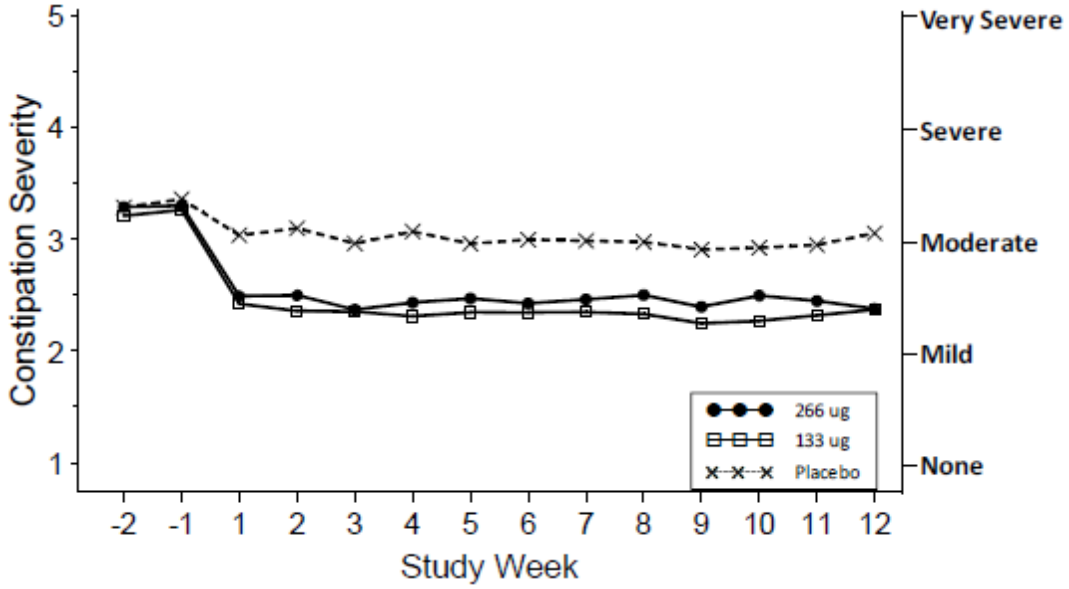
Visit	Statistic	Placebo (N=209)	Linaclotide 133 ug (N=217)	Linaclotide 266 ug (N=216)
Baseline	Mean	3.321	3.248	3.311
	SD	0.730	0.779	0.725
	SEM	0.051	0.053	0.050
	Median	3.000	3.000	3.250
	Min, Max	1.25, 5.00	1.00, 5.00	1.00, 5.00
	n	209	213	210
Treatment Overall	Mean	2.997	2.338	2.452
	SD	0.760	0.803	0.825
	SEM	0.053	0.055	0.057
	Median	3.000	2.333	2.417
	Min, Max	1.08, 5.00	1.00, 5.00	1.00, 5.00
	n	209	213	210
ANCOVA Results	LS Mean Change from Baseline (SE)	-0.271 (0.053)	-0.897 (0.053)	-0.810 (0.053)
	LS Mean Difference (95% CI) [Linaclotide - Placebo]		-0.626 (-0.76, -0.49)	-0.539 (-0.68, -0.40)
	P-value[a]		< 0.0001	< 0.0001

Data source: Section 14, Table 14.4.2.5A
Constipation Severity was measured weekly using a five-point ordinal scale (1 = none; 2 = mild; 3 = moderate; 4 = severe; 5 = very severe). The patient's Constipation Severity score for the Treatment Period is the average of the nonmissing weekly patient assessments of Constipation Severity scores reported during the 12-week Treatment Period.
n = Number of patients with analysis values at both baseline and a specific time point in the ITT Population.
SD=standard deviation, SEM=standard error of the mean, Min=minimum, Max=maximum and SE = Standard Error of LS Mean.
[a] P-values are based on a pairwise comparison versus placebo in an ANCOVA model with treatment group and geographic region factors and baseline value as covariate. P-values are less than the threshold value for statistical significance based on the MCP.

As seen from the table above, the LS mean change from baseline in abdominal discomfort for the linaclotide 133 µg and 266 µg groups were slightly numerically greater than placebo, and the difference was statistically significant for both linaclotide doses compared to placebo.

Mean constipation severity during the treatment period is graphed below by week.

Mean Constipation Severity (OC) by Week (Treatment Period)
ITT Population
MCP-103-303



Copied from Figure 12.

As seem from the figure above, both the 133- and 266-µg/day doses demonstrated a separation from placebo that was observed during Week 1 and sustained across the 12-week treatment period.

3.1.2.3 Reviewer's Comments and Evaluation

3.1.2.3.1 IVR Call

The proportion of patients who had completed at least 80% IVRS calls for the first 12 weeks of treatment for the placebo was slightly higher that for linaclotide 133 µg and 266 µg patients (84.2% vs. 80.2% and 77.4%).

The proportion of patients who had completed at least 4 IVRS calls for at least 9 of the first 12 weeks of treatment for the linaclotide 266 µg subjects was slightly lower than those of the placebo and the linaclotide 133 µg subjects (82.9% vs. 85.2% and 86.2%).

The proportion of patients who had completed at least 4 IVRS calls for all of the first 12 weeks of treatment for the linaclotide 266 µg subjects was slightly lower than those of the placebo and the linaclotide 133 µg subjects (67.1% vs. 75.1% and 75.1%).

3.1.2.3.2 Modified Intent-to-Treat Analysis of Primary Efficacy Endpoint

The sponsor performed modified ITT analysis on the primary efficacy endpoint where a subject with fewer than 4 complete IVRS calls in a Treatment Period week

was considered a nonresponder for that week. The primary 12-week CSBM overall responder endpoint was then calculated based on the CSBM Weekly Responder endpoints.

The results from modified ITT analyses of 12 week CSBM overall responder are given below.

12-Week CSBM Overall Responders (Modified)
ITT Population
Study MCP-103-303

Description	Placebo (N=209) n (%)	LIN 133 ug (N=217) n (%)	LIN 266 ug (N=216) n (%)
Responder	7 (3.3)	44 (20.3)	41 (19.0)
Non-responder	202 (96.7)	173 (79.7)	175 (81.0)
Difference in Responder Rate (Linaclotide - Placebo)		16.9	15.6
Odds Ratio for Response (Linaclotide : Placebo)		7.25	6.97
95% C.I. for odds ratio		(3.20, 16.42)	(3.03, 16.05)
P-value		<.0001	<.0001

Notes: A 12-week CSBM overall responder (modified) is a patient who is a CSBM weekly responder (modified) for at least 9 of the 12 weeks of the Treatment Period. A CSBM weekly responder (modified) is a patient who had a CSBM weekly frequency rate that was 3 or greater, increased by 1 or more from baseline and who completed at least 4 IVRS calls for that week.
n = Number of patients within a specific category. N = Number of patients in the ITT Population.
Odds ratio, 95% CI (Confidence Interval) and p-values were obtained from the CMH tests controlling for geographic region, comparing each linaclotide dose versus placebo in a pairwise manner.

As seen from the table above, the results were similar to those from sponsor's the original analysis. Only slight changes on number of responders different from the original analysis (0, 2 and 1 less responders for placebo, linaclotide 133 µg, and linaclotide 266 µg, respectively) in the revised analysis as compared to the original analysis were observed.

3.1.2.3.3 Sensitivity Analyses of 12-Week CSBM Overall Responders

Per request, the sponsor performed the several sensitivity analyses of 12-week CSBM overall responder.

The results from sensitivity analyses of f 12-week CSBM overall responder are given below.

12-week CSBM Overall Responders
Study MCP-103-303

Analysis	PLA	LIN 133 µg	Diff	LIN 266 µg	Diff
(LOCF)	9/209 (4.3%)	54/217 (24.9%)	20.6%	47/216 (21.8%)	17.5%
Completed Case	6/157 (3.8%)	40/163 (24.5%)	20.7%	31/145 (21.4%)	17.6%
Observed Case	7/209 (3.3%)	44/216 (20.4%)	17.1%	41/215 (19.1%)	15.8%
Worst Case 1	6/209 (2.9%)	40/217 (18.4%)	15.5%	31/216 (14.4%)	11.5%
Worst Case 2	58/209 (27.8%)	40/217 (18.4%)	-9.4%	31/216 (14.4%)	-13.4%
Worst Case 3	18.209 (8.6%)	44/217 (20.3%)	11.7%	41/216 (19.0%)	10.4%
Multiple Imputation	3.9%	23.8%	19.9%	21.8%	17.9%

Complied from Tables 14.4.1.1B, 14.4.1.1.D-14.4.1.1H
P- values were obtained from the CMH tests controlling for geographic region.
The complete case analysis includes only those patients who complete at least 4 IVRS calls for each of the first 12 weeks of treatment.
The observed case analysis includes only those patients who complete at least 4 IVRS calls for at least one of the first 12 weeks of treatment.
For worst case analysis 1, patients must complete at least 4 IVARS calls for each of the first 12 weeks of treatment.
For worst case analysis 2, patients who do not complete at least 4 IVRS call for each of the first 12 weeks of treatment are handled as follows: patients randomized to Linaclotide are non-responders, while patients who are randomized to placebo are considered responders.
For worst case analysis 3, for those weeks where patients do not complete at least 4 IVRS calls, patients randomized to Linaclotide are non-responders, while patients who are randomized to placebo are considered responders.

The sponsor applied the following definition for Worst Case 1:

- Worst Case 1: If a patient has less than 4 complete calls for any of the first 12 Treatment Period weeks, that patient was assumed to be "failed" and defined as a nonresponder for the trial.

Under the Worst Case 1 method, if a patient had less than 4 complete IVRS calls in any one of Treatment Period weeks 1 - 12, that patient was defined as a primary efficacy endpoint nonresponder.

In contrast, for the modified analysis for the primary efficacy endpoint in the CC trials if a patient had less than 4 complete IVRS calls for one or more of the Treatment Period weeks 1 - 12, that patient would be defined as a weekly nonresponder for those particular weeks, but could still be a primary efficacy endpoint responder.

As such, the number of patients classified as primary efficacy endpoint responders under the Worst Case 1 method was lower than that under modified analysis for the primary efficacy as only those patients who have at least 4 complete IVRS calls in all

12 Treatment Period weeks could potentially be primary efficacy endpoint responders under the Worst Case 1 method. As compared with the modified analysis, Worst Case analysis would yield 1, 4 and 10 less responder for placebo, linaclotide 133 µg and linaclotide 266 µg, respectively..

The sponsor's Worst Case 1 analysis is one of "worst cast" analyses. It was more conservative than the sponsor's modified analysis.

As seen from the table above, for the 12- week CSBM overall responder, it was shown by a significantly greater proportion of subjects taking either linaclotide 133 µg or linaclotide 266 µg compared with subjects taking placebo in all sensitivity analyses except the Worst Case 2 analysis.

Treatment difference between the linaclotide 266 µg and the linaclotide 133 µg was small; it ranged from -4.0% to -1.3%.

3.1.2.3.4 Subgroup Analysis of 12-Week CSBM Overall Responder

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 12-week CSBM overall responders for gender, age, race, region, BMI at baseline.

A summary of the results of the subgroup analyses of proportion of 12-week CSBM overall responders is given in the Appendix Table 7.

As seen from Appendix Table 7, 12-week CSBM overall responder were reported by higher proportion of linaclotide subjects for gender, age <65, race, and BMI ≥30 kg/m^2 at baseline, abdominal pain at baseline (≥ 5 < 8).

3.1.2.3.5 Weekly CSBM Responder Rates by Week

As per request, the sponsor provided observed case analysis of number of subjects with weekly CSBM responder by week (see below).

CSBM Weekly Responder Rate by Treatment Group
Observed Case
Study MCP-103-303

	PLA	LIN 133	Diff (LIN 133-PLA)	LIN 266	Diff (LIN 266 – PLA)
Week 1	16/209 (7.7%)	82/214 (38.3%)	30.7%	70/213 (32.9%)	25/2%
Week 2	21/206 (10.2%)	73/212 (34.4%	24.2%	76/202 (37.6%)	27.4%
Week 3	27/200 (13.5%)	79/205 (38.5%)	25.0%	76/200 (38.0%)	24.5%
Week 4	25/199 (12.6%)	84/202 (41.6%)	29.0%	63/195 (32.3%)	19.7%
Week 5	24/189 (12.7%)	76/197 (38.6%)	25.9%	68/191 (35.6%)	22.9%
Week 6	26/186 (14.0%)	73/190 (38.4%)	24.4%	69/189 (36.5%)	22.5%
Week 7	23/181 (12.6%)	75/192 (39.1%)	26.4%	65/183 (35.5%)	22.9%
Week 8	27/183 (14.8%)	65/187 (34.8%)	20.0%	63/176 (35.8%)	21.0%
Week 9	24/175 (13.7%)	68/184 (37.0%)	23.2%	67/176 (38.1%)	24.4%
Week 10	29/175 (16.6%)	68/183 (37.2%)	23.2%	62/180 (34.4%)	24.4%
Week 11	25/175 (14.3%)	74/180 (41.1%)	26.8%	61/172 (35.5%)	21.2%
Week 12	19/172 (11.0%)	63/183 (34.4%)	23.4%	49/171 (28.7%)	17.6%

Compiled by this reviewer from Table 14.4.1.1L.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, greater proportions of subjects at almost every week during the course of the 12- week study in the linaclotide group compared with subjects in the placebo group was observed.

Treatment difference between the linaclotide 266 µg and the linaclotide 133 µg varied by week. At Week 12, the low dose was 5.8% higher than the high dose.

3.1.2.3.6 Monthly CSBM Responder Rate

The monthly CSBM responder is defined that a subject be a CSBM weekly responder for at least 3 of the 4 treatment period weeks for that month. A subject with missing monthly responder at specific month was considered non-responder for that month.

This reviewer performed analyses of the monthly CSBM responder by month.

The results from reviewer's analyses of the monthly CSBM responder by month are given below.

Monthly CSBM Responder Rate by Treatment Group
Intention-to-Treat Population
Study MCP-103-303

	PLA	LIN 133	Diff (LIN 133-PLA)	LIN 266	Diff (LIN 266 – PLA)
Month 1	17/209 (8.1%)	62/217 (28.6%)	20.5%	54/217 (24.9%)	16.8%
Month 2	12/209 (5.7%)	60/217 (27.6%)	20.8%	54/217 (24.9(%)	19.2%
Month 3	14/209 (6.7%)	56/217 (25.8%)	19.1%	43/217 (19.8%)	13.1%

Obtained by this reviewer using the sponsor's weekly CSBM data.

As seen from the tables above, for monthly CSBM responder, greater proportions of subjects at every month during the course of the 3-month study in each linaclotide group compared with subjects in the placebo group was observed.

The linaclotide 133 μg was numerically higher than the linaclotide 266 μg at Month 1 and Month 3. But, treatment difference ranged from 1.6% to 6.0%.

3.1.2.3.7 Sustained Efficacy – All 3 Months

For sustained efficacy, the commonly used endpoint for CC is the "overall responder." A subject was considered an overall responder if the subject was a month responder for all three months during 12-week study.

This "overall responder" based on monthly responders is more stringent than the pre-specified overall responder based on weekly responders.

This reviewer performed analysis of overall CSBM responder for monthly CSBM responders.

The results are given below.

Reviewer's "Overall" CSBM Responder Analysis by Treatment Group
Intention-to-Treat Population
Study MCP-103-303

PLA	LIN 133	Diff (LIN 133-PLA)	LIN 266	Diff (LIN 266 – PLA)
5/209 (2.4%)	27/217 (12.4%)	10.0%	25/217 (11.5%)	9.1%

Obtained by this reviewer using the sponsor's weekly CSBM data.

As seen from the tables above, for overall CSBM responder for monthly CSBM responder, greater proportions of subjects during the course of the first 12-week treatment period study in each linaclotide group compared with subjects in the placebo group was observed.

The results for both doses were similar each other.

3.1.2.3.8 Sustained Efficacy – 9 of 12 Weeks and 3 of 4 Weeks at Month 3

For sustained efficacy, the other endpoint recommended recently for CC is the "overall responder." A subject was considered an overall responder if the subject was a week responder for at least of 9 of 12 weeks and at least 3 of months at Month 3 during the 12-week treatment period.

This reviewer performed analysis of overall CSBM responder for monthly CSBM responders.

The results are given below.

Reviewer's "Overall" CSBM Responder Analysis by Treatment Group
Intention-to-Treat Population
Study MCP-103-303

PLA	LIN 133	Diff (LIN 133-PLA)	LIN 266	Diff (LIN 266 – PLA)
6/209 (2.9%)	35/217 (16.1%)	13.2%	37/217 17.1%)	14.2%

Obtained by this reviewer using the sponsor's weekly CSBM data.

As seen from the tables above, for overall CSBM responder for monthly CSBM responder, greater proportions of subjects during the course of the first 12-week treatment period study in each linaclotide group compared with subjects in the placebo group was observed.

The results for both doses were similar each other.

3.1.2.3.9 Reviewer's Comments on Sponsor's Controlling for Multiplicity for Primary and Secondary Efficacy Parameter

The sponsor used 5-step serial gatekeeping multiple comparison procedure to control type 1 family-wise error rate for testing the primary and secondary efficacy parameters.

The detailed procedure is listed below.

The overall type I family-wise error rate for testing the primary and secondary efficacy parameters was controlled at the 0.05 significance level using the following 5-step serial gatekeeping multiple comparisons procedure (MCP). Following this MCP, progression to the next step only occurred if all individual hypotheses within a step were rejected and the previous step(s) were all rejected at the step-specific overall significance level. If all hypotheses within a step were not rejected, the hypothesis tests involved in all subsequent steps were considered not statistically significant. All hypothesis tests were 2-sided.

1. The first step tested the primary efficacy parameter for the 266 µg group at the 0.05 significance level
2. The second step tested the primary efficacy parameter for the 133 µg group and the first 5 secondary parameters (i.e., CSBM Frequency, SBM frequency, Stool Consistency, Severity of Straining, and Constipation Severity) for the 266 µg group. The 6 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters.
3. The third step tested the last 2 secondary efficacy parameters (i.e., Bloating and Abdominal Discomfort) for the 266 µg group. The 2 individual

hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters.

4. The fourth step tested the first 5 secondary efficacy parameters for the 133 µg group. The 5 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters.
5. The fifth step tested the last 2 secondary efficacy parameters for the 133 µg group. The 2 individual hypotheses within this step were tested using an overall type I error rate of 0.05 by means of a Hochberg procedure to control for multiple parameters.

This reviewer's comments on this gatekeeping procedure were

> The sponsor's gatekeeping procedure was not appropriate. The Hochberg procedure is generally not recommended for sequential testing. It is not assumption free. Furthermore, it is known to provide overall α-control for independent and for certain types of positive correlated endpoints. But its properties for other types of dependent endpoints are not fully known. Various simulation experiments indicate that this method generally controls the overall Type 1 error rate for positive correlated endpoints but fails to do so for some negatively correlated endpoints.
>
> The sponsor should use a Bonferroni based gatekeeping procedure to test all endpoints in the primary family and proceed to the secondary family of endpoints only if there has been statistical success in the primary family.

Furthermore, since p-values for most secondary endpoints were very small (<0.001), all secondary endpoints would pass any statistical procedure for controlling the type 1 error for multiplicity.

3.1.2.3.10 Reviewer's Comments on Results of Secondary Efficacy Endpoints

The sponsor's pre-specified analysis for the secondary endpoints was based on a modeling approach (ANCOVA) using all data for each week 1-12. The term "treatment overall" refers to an average treatment effect over the 12 weeks of the study. (b) (4)

The sponsor's the summary of secondary efficacy endpoints is given below.

Summary of Secondary Efficacy Endpoints
Study MCP-103-303
(ITT Population)

Parameter	*Mean Baseline*	*Placebo (N = 209) LS Mean Change (SE)*	*Linaclotide*			
			145 ug (N = 217)		*290 ug (N = 216)*	
			LS Mean Change (SE)	**LSMD (95% CI)**	**LS Mean Change (SE)**	**LSMD (95% CI)**
CSBMs/Week	0.3	0.5 (0.2)	1.9[c] (0.2)	1.5 (1.0, 1.9)	2.0[c] (0.2)	1.6 (1.2, 2.0)
SBMs/Week	2.1	1.1 (0.2)	3.0[c] (0.2)	2.0 (1.4, 2.5)	3.0[c] (0.2)	1.9 (1.4, 2.5)
Stool Consistency (BSFS Score)	2.4	0.6 (0.1)	1.9[c] (0.1)	1.3 (1.1, 1.5)	1.8[c] (0.1)	1.3 (1.0, 1.5)
Severity of Straining (5-point Ordinal Scale)	3.2	-0.5 (0.1)	-1.1[c] (0.1)	-0.6 (-0.7, -0.5)	-1.2[c] (0.1)	-0.6 (-0.8, -0.5)
Abdominal Discomfort (5-point Ordinal Scale)	2.5	-0.3 (0.0)	-0.5[b] (0.0)	-0.2 (-0.3, -0.1)	-0.4[a] (0.0)	-0.1 (-0.2, 0.0)
Bloating (5-point Ordinal Scale)	2.8	-0.2 (0.0)	-0.5[c] (0.0)	-0.2 (-0.3, -0.1)	-0.4[a] (0.0)	-0.2 (-0.3, -0.1)
Constipation Severity (5-point Ordinal Scale)	3.3	-0.3 (0.1)	-0.9[c] (0.1)	-0.6 (-0.8, -0.5)	-0.8[c] (0.1)	-0.5 (-0.7, -0.4)

The mean change from baseline is a least-squares mean change based on an ANCOVA model with treatment group and geographic region as factors and baseline value as covariate.

Baseline is the mean value for the combined ITT Population.

CI = confidence interval; LS = Least squares; LSMD = least squares mean difference; SE = standard error of LS mean.

p-values based on a pairwise comparison versus placebo in an ANCOVA model.

a. $p \leq 0.05$
b. $p < 0.001$
c. $p < 0.0001$

Source: MCP-103-303 Tables 14.2.4 and 14.4.2.1A to 14.4.2.7A

As seen from the table above, secondary efficacy endpoints were statistically significantly improved for both doses of linaclotide compared with placebo. However, treatment differences for changes from base for severity of straining, abdominal discomfort, bloating and constipation severity might not be clinically meaningful.

The results for both doses were similar to each other.

Per request, the sponsor also performed sensitivity analyses for secondary efficacy endpoints. The results are summarized in the Appendix Table 8.

As seen from Appendix Table 8, all sensitivity analyses (LOCF, CC, OC, BOCF and MI) gave similar results.

3.1.3 Pooled Efficacy Analysis

3.1.3.1 12-Week CSBM Overall Responder

This reviewer performed analysis for primary efficacy endpoint, 12-week CSBM overall responder, for sponsor's ITT and modified ITT populations pooling studies LIN-MD-01 and MCP-103-303.

Summary of the results from the pooled analysis is given below.

12-week CSBM Overall Responders
Pooled Studies LIN-MD-01 and MCP-103-303

Analysis	PLA	LIN 133 µg	Diff	LIN 266 µg	Diff
ITT	20/424 (4.7%)	80/430 (18.6%)	13.9%	85/418 (20.3%)	15.6%
Modified ITT	19/424 (4.5%)	77/430 (17.9%)	13.4%	82/418 (19.6%)	15.1%

Complied by the reviewer.

Per request, the sponsor performed sensitivity analyses for primary efficacy endpoint, 12-week CSBM overall responder, for pooled ITT population.

Summary of sensitivity analyses for primary efficacy endpoint is given below.

12-week CSBM Overall Responders
Pooled Studies LIN-MD-01 and MCP-103-303

Analysis	PLA	LIN 133 µg	Diff	LIN 266 µg	Diff
(LOCF)	23/423 (5.4%)	101/430 (23.5%)	18.1%	98/418 (23.4%)	18.0%
Completed Case	17/306 (5.6%)	69/299 (23.1%)	17.5%	65/272 (23.9%)	18.3%
Observed Case	19/423 (4.5%)	77/425 (18.1%)	13.6%	82/415 (19.8%)	15.3%
Worst Case 1	17/423 (4.0%)	69/430 (16.0%)	12.0%	65/418 (15.6%)	11.5%
Worst Case 2	134/423 (31.7%)	69/430 (16.0%)	-15.6%	65/418 (15.6%)	-16.1%
Worst Case 3	47/423 (11.1%)	77/430 (6.8%)	6.8%	82/418 (19.6%)	8.5%
Multiple Imputation	5.0%	22.8%	17.8%	22.4%	19.2%

Complied from Tables 3.1.1R.1-7

As seen from the tables above, for the 12-week CSBM overall responders, it was shown by a significantly greater proportion of subjects taking either linaclotide 133 µg or linaclotide 266 µg compared with subjects taking placebo in sponsor's ITT and modified ITT analysis and all sensitivity analyses except the Worst Case 2 analysis.

The results for both doses were similar each other.

3.1.3.2 CSBM Weekly Responder

Per request, the sponsor performed pooled analyses for CSBM weekly responder by week for pooled ITT population.

Summary of pooled analyses for CSBM weekly responder by week for pooled ITT population is given below.

CSBM Weekly Responder Rate by Treatment Group
ITT Population
Pooled Studies LIN-MD-01 and MCP-103-303

	PLA	LIN 133	Diff (LIN 133-PLA)	LIN 266	Diff (LIN 266 – PLA)
Week 1	38/423 (9.0%)	149/430 (34.7%)	25.7%	148/418 (35.4%)	26.4%
Week 2	54/423 (12.8%)	133/430 (30.9%)	18.2%	154/418 (36.8%)	24.1%
Week 3	50/423 (11.8%)	141/430 (32.8%)	21.0%	151/418 (36.1%)	24.3%
Week 4	58/423 (13.7%)	150/430 (34.9%)	21.2%	153/418 (36.6%)	22.9%
Week 5	49/423 (11.6%)	138/430 (32.1%)	20.5%	146/418 (34.9%)	23.3%
Week 6	51/423 (12.1%)	134/430 (31.2%)	19.1%	143/418 (34.2%)	22.2%
Week 7	54/423 (12.8%)	141/430 (32.8%)	20.0%	135/418 (32.3%)	19.5%
Week 8	58/423 (13.7%)	118/430 (27.4%)	13.7%	136/418 (32.5%)	18.8%
Week 9	51/423 (12.1%)	119/430 (27.7%)	15.6%	133/418 (31.8%)	19.8%
Week 10	53/423 (12.5%)	125/430 (29.1%)	16.5%	132/418 (31.6%)	19.0%
Week 11	52/423 (12.3%)	128/430 (29.8%)	17.5%	127/418 (30.4%)	18.1%
Week 12	52/423 (12.3%)	115/430 (26.7%)	14.5%	102/418 (24.4%)	12.1%

Compiled by this reviewer from Table 3.1-10.
P-values were obtained by the CMH tests controlling for geographic region.

As seen from the table above, treatment difference between the linaclotide 266 µg and the linaclotide 133 µg varied by week. The results for both doses were similar each other.

3.2 Evaluation of Safety

3.2.1 Study LIN-MD-01

The only TEAEs experienced by at least 3% of linaclotide patients, and at an incidence at least 1% above that of placebo patients were diarrhea (17.2% vs. 2.8%) and abdominal pain (5.3% vs. 2.3%). There was no apparent relationship between linaclotide dose and TEAE incidence.

Most of the TEAEs were reported as mild or moderate. Of the 587 TEAEs reported in linaclotide patients 40 (6.8%) were judged to be severe, compared with 16 (6.0%) of 268 TEAEs in placebo patients.

Diarrhea was the most frequently reported TEAE among linaclotide patients; 42 (19.7%) patients in the linaclotide 133-µg/day group and 30 (14.6%) patients in the linaclotide 266-µg/day group experienced at least 1 episode of diarrhea vs. only 6

(2.8%) placebo patients. A total of 7 of the 72 linaclotide patients who experienced TEAEs of diarrhea had events that were reported as severe. A total of 22 (5.3%) linaclotide patients discontinued from the study because of a TEAE of diarrhea versus only 1 (0.5%) placebo patient.

Per this reviewer's request, the sponsor performed analysis of number of patients with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea.

The results are given below.

Number of Patients with at Least One AE, at Least One TRAE. Withdrawn due to AE, at Least One Episode of Diarrhea, and Discontinued due to TRAE of Diarrhea
Study LIN-MD-01

Parameter	Placebo (N=215) n (%)	LIN 145 ug (N=213) n (%)	LIN 290 ug (N=205) n (%)	Odds Ratio [Exact 95% CI for Odds Ratio (Lin 145 : Placebo)]	Odds Ratio [Exact 95% CI for Odds Ratio (Lin 290 : Placebo)]
Number of Patients with at Least One AE	117 (54.4)	140 (65.7)	116 (56.6)	1.61 (1.07 , 2.42)	1.09 (0.73 , 1.63)
p-value		0.0181	0.6948		
Number of Patients with at Least One TRAE	32 (14.9)	68 (31.9)	48 (23.4)	2.68 (1.63 , 4.45)	1.75 (1.04 , 2.97)
p-value		<.0001	0.0342		
Number of Patients Withdrawn due to AE	10 (4.7)	20 (9.4)	19 (9.3)	2.12 (0.92 , 5.21)	2.09 (0.90 , 5.17)
p-value		0.0601	0.0822		
Number of Patients with at Least One Episode of Diarrhea	6 (2.8)	42 (19.7)	30 (14.6)	8.56 (3.49 , 25.11)	5.97 (2.36 , 17.89)
p-value		<.0001	<.0001		
Number of Patients Discontinued due to TRAE of Diarrhea	1 (0.5)	11 (5.2)	10 (4.9)	11.65 (1.66 ,503.89)	10.97 (1.53 ,478.43)
p-value		0.0029	0.0048		

Copied from Table 9.1

As seen from the table above, for number of patients with at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea, greater proportions of subjects in the linaclotide group compared with subjects in the placebo group was observed.

3.2.2 Study MCP-103-303

The percentage of patients experiencing TEAEs was comparable in each group, with 105 patients (50.2%), 122 patients (56.2%), and 119 patients (54.8%) in the placebo, 133 µg, and 266 µg dose groups, respectively, experiencing at least 1 TEAE. As for SAEs, 5 patients (2.4%), 3 patients (1.4%), and 4 patients (1.8%) in the placebo, 133 µg linaclotide, and 266 µg linaclotide groups, respectively, experienced an SAE. More patients discontinued from study drug due to a TEAE in the linaclotide groups compared to placebo, with 8 patients (3.8%), 11 patients (5.1%), and 11 patients (5.1%) in the placebo, 133, and 266 µg dose groups, respectively, experiencing TEAEs that led to discontinuation. There were no deaths reported in this trial.

The incidence of patients reporting TEAEs was comparable for placebo-treated patients (50.2%) and all linaclotide-treated patients (55.5%). The most common SOCs (in which ≥ 10% of patients for any treatment group reported TEAEs) were Gastrointestinal Disorders (21.5% of placebo patients, 27.2% of linaclotide patients)

and Infections and Infestations (14.4% of placebo patients, 17.5% of linaclotide patients). In general, there were few noteworthy differences in the numbers of patients who reported specific TEAEs between placebo and the linaclotide groups, with the most prominent exception being the most common TEAE observed during the trial – diarrhea, reported by 14 (6.7%) of 209 placebo patients and 57 (13.1%) of 434 of linaclotide patients. The incidence of diarrhea was similar between the linaclotide 133 µg (12.4%) and the 266 µg (13.8%) dose groups.

Fourteen patients (6.7%) in the placebo group had diarrhea. Diarrhea was reported in 27 patients (12.4%) in the 133 µg linaclotide group and 30 patients (13.8%) in the 266 µg linaclotide dose group. Three patients (1.4%) in each linaclotide dose group had severe diarrhea, and 1 patient (0.5%) in the placebo group had severe diarrhea.

Per this reviewer's request, the sponsor performed analysis of number of patients with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea.

The results are given below.

Number of Patients with at Least One AE, at Least One TRAE, Withdrawn due to AE, at Least One Episode of Diarrhea, and Discontinued due to TRAE of Diarrhea
Study MCP-103-303

Parameter	Placebo (N=209) n (%)	LIN 145 ug (N=217) n (%)	LIN 290 ug (N=217) n (%)	Odds Ratio [Exact 95% CI for Odds Ratio (Lin 145 : Placebo)]	Odds Ratio [Exact 95% CI for Odds Ratio (Lin 290 : Placebo)]
Number of Patients with at Least One AE	106 (50.7)	122 (56.2)	119 (54.8)	1.25 (0.84 , 1.86)	1.18 (0.79 , 1.76)
p-value		0.2854	0.4376		
Number of Patients with at Least One TRAE	30 (14.4)	47 (21.7)	44 (20.3)	1.65 (0.97 , 2.83)	1.52 (0.89 , 2.62)
p-value		0.0588	0.1249		
Number of Patients Withdrawn due to AE	8 (3.8)	12 (5.5)	11 (5.1)	1.47 (0.54 , 4.24)	1.34 (0.48 , 3.92)
p-value		0.4946	0.6411		
Number of Patients with at Least One Episode of Diarrhea	14 (6.7)	27 (12.4)	30 (13.8)	1.98 (0.97 , 4.21)	2.23 (1.11 , 4.70)
p-value		0.0494	0.0170		
Number of Patients Discontinued due to TRAE of Diarrhea	1 (0.5)	8 (3.7)	6 (2.8)	7.96 (1.05 ,354.94)	5.91 (0.71 ,273.34)
p-value		0.0374	0.1224		

Copied from Table 9-2.

As seen from the table above, for number of patients with at least one episode of diarrhea, and discontinued due to TRAE of diarrhea, greater proportions of subjects in each linaclotide group compared with subjects in the placebo group was observed.

3.2.3 Pooled Analysis

Per this reviewer's request, the sponsor performed pooled analysis of number of patients with at least one AE, at least one treatment related AE (TRAE), withdrawn due to AE, at least one episode of diarrhea, and discontinued due to TRAE of diarrhea.

The results are given below.

Number of Patients with at Least One AE, at Least One TRAE, Withdrawn due to AE, at Least One Episode of Diarrhea, and Discontinued due to TRAE of Diarrhea
Pooled Studies LIN-MD-01 and MCP-103-303

Parameter	Placebo (N=423) n (%)	LIN 145 ug (N=430) n (%)	LIN 290 ug (N=422) n (%)	Odds Ratio [Exact 95% CI for Odds Ratio (Lin 145 : Placebo)]	Odds Ratio [Exact 95% CI for Odds Ratio (Lin 290 : Placebo)]
Number of Patients with at Least One AE	222 (52.5)	262 (60.9)	235 (55.7)	1.41 (1.07 , 1.87)	1.14 (0.86 , 1.51)
p-value		0.0130	0.3697		
Number of Patients with at Least One TRAE	62 (14.7)	115 (26.7)	92 (21.8)	2.13 (1.49 , 3.05)	1.62 (1.12 , 2.36)
p-value		<.0001	0.0075		
Number of Patients Withdrawn due to AE	18 (4.3)	32 (7.4)	30 (7.1)	1.81 (0.97 , 3.48)	1.72 (0.91 , 3.34)
p-value		0.0576	0.0764		
Number of Patients with at Least One Episode of Diarrhea	20 (4.7)	69 (16.0)	60 (14.2)	3.85 (2.26 , 6.82)	3.34 (1.94 , 5.96)
p-value		<.0001	<.0001		
Number of Patients Discontinued due to TRAE of Diarrhea	2 (0.5)	19 (4.4)	16 (3.8)	9.73 (2.32 , 86.52)	8.30 (1.93 , 74.69)
p-value		0.0002	0.0006		

Copied from Table 9.5

As seen from the table above, for number of patients with at least one episode of diarrhea, and discontinued due to TRAE of diarrhea, greater proportions of subjects in each linaclotide group compared with subjects in the placebo group was observed.

4. FINDINGS IN SPECIAL/SUBGROUP POPULATION

4.1 Gender, Race and Age

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 12-week CSBM overall responders for gender, age, and race, and BMI at baseline.

4.1.1 Study LIN-MD-31

The summary of results of subgroup analyses of proportion of 12-week CSBM overall responders for Study LIN-MD-31 is given below.

Subgroup Analyses of Proportion of 12-Weelk CSBM Overall Responders
Study LIN-MD-01

Subgroup	Placebo	Linaclotidde 133 ug	Difference (LIN133-PLA)	95% CI	Linaclotidde 266 ug	Difference (LIN266-PLA)	95% CI
Gender							
Male	1/19 (5.3%)	3/18 (16.7%)	11.4%	(8.5%, 31.3%)	9/23 (39.1%)	33.8%	(11.5%, 56.2%)
Female	12/196 (6.1%)	31/195 (15.9%)	9.8%	(3.6%, 15.9%)	34/179 (19.0%)	12.9%	(6.2%, 19.5%)
Age							
<65	11/188 (5.9%)	27/189 (14.3%)	8.4%	(2.4%, 14.4%)	38/181 (21.0%)	15.1%	(8.3%, 22.0%)
≥65	2/27 (7.4%)	7/24 (29.2%)	21.8%	(1.1%, 42.4%)	5/21 (23.8%)	16.4%	(-4.3%, 37.1%)
Race							
White	8/168 (4.8%)	29/168 (17.3%)	12.5%	(5.9%, 19.1%)	38/152 (25.0%)	20.2%	(12.6%, 27.8%)
Black	5/42 (11.9%)	4/41 (9.8%)	-2.1%	(-15.5%, 11.2%)	4/46 (8.7%)	-3.2%	(-15.9%, 9.5%)
Other	0/5 (0.0%)	1/4 (25.0%)	25.0%	(-17.4%, 67.4%)	1/4 (25.0%)	25.0%	(-17.4%, 67.4%)

Compiled by this reviewer from Table 14.4.1.1I.1- 14.4.1.1I-5, and 14.4.1.1M

As seen from the table above, 12-week CSBM overall responder rates were numerically higher for linaclotide subjects for gender, age and race.

4.1.2 Study MCP-103-303

A summary of the results of the subgroup analyses of proportion of 12-week CSBM overall responders for Study MCP-103-302 is given below.

Subgroup Analyses of Proportion of 12-Weelk CSBM Overall Responders Study MCP-103-302

Subgroup	Placebo	Linaclotidde 133 ug	Difference (LIN133-PLA)	95% CI	Linaclotidde 266 ug	Difference (LIN266-PLA)	95% CI
Gender							
Male	1/27 (3.7%)	10/26 (38.5%)	34.8%	(14.5%, 54.7%)	6/28 (21.4%)	17.7%	(0.7%, 34.5%)
Female	6/182 (3.3%)	36/191 (18.8%)	15.5%	(9.4%, 21.7%)	36/188 (19.1%)	16.3%	(9.6%, 22.0%)
Age							
<65	6/181 (3.3%)	36/190 (18.9%)	15.6%	(9.5%, 21.8%)	37/189 (19.6%)	16.3%	(10.0%, 22.5%)
≥65	1/28 (3.6%)	10/27 (37.0%)	33.5%	(14.0%, 52.9%)	5/27 (18.5%)	16.4%	(-1.2%, 31.1%)
Race							
White	3/160 (1.9%)	37/164 (22.6%)	20.7%	(14.0%, 27.4%)	32/157 (20.4%)	18.5%	(11.9%, 25.2%)
Black	4/46 (8.7%)	9/46 (19.6%)	10.9%	(-7.2%, 26.8%)	8/52 (15.4%)	6.7%	(-6.1%, 19.4%)
Other	0/3 (0.0%)	0/7 (0.0%)	0.0%		2/7 (28.6%)	28.6%	(-4.9%, 62.0%)

Compiled by this reviewer from Table 14.4.1.1I.1- 14.4.1.1I-5, and 14.4.1.1M

As seen from the table above, 12-week CSBM overall responders rates were numerically higher for linaclotide subjects for gender, age and race.

4.2 Other Special/Subgroup Population

Per this reviewer's request, the sponsor performed the subgroup analyses of proportion of 12-week CSBM overall responders for regions, and BMI at baseline

4.2.1 Study LIN-MD-01

The summary of results of subgroup analyses of proportion of 12-week CSBM overall responders for Study LIN-MD-01 is given below.

Subgroup Analyses of Proportion of 12-Wweek CSBM Overall Responders Study LIN-MD-01

Subgroup	Placebo	Linaclotidde 133 ug	Difference (LIN133-PLA)	95% CI	Linaclotidde 266 ug	Difference (LIN266-PLA)	95% CI
Region							
Canada	0/7 (0.0%)	0/5 (0.0%)	0.0%		1/7 (14.3%)	14.3%	(-11.6%, 40.2%)
Midwest	1/32 (3.1%)	5/25 (20.0%)	16.9%	(0.0%, 33.7%)	9/29 (31.0%)	26.9%	(10.0%, 45.8%)
Northeast	1/18 (5.6%)	2/13 (15.4%)	9.8%	(-12.5%, 32.1%)	4/11 (36.4%)	30.8%	(0.5%, 61.1%)
Southeast	8/98 (8.2%)	18/100 (18.0%)	9.8%	(0.6%, 19.1%)	19/96 (19.8%)	11.6%	(2.0%, 21.3%)
Southwest	1/26 (3.8%)	6/32 (18.8%)	15.0%	(-0.5%, 30.3%)	6/30 (20.0%)	16.2%	(0.0%, 32.3%)
West	2/34 (5.9%)	3/38 (7.9%)	2.0%	(-9.7%, 13.7%)	4/29 (13.8%)	7.9%	(-6.9%), 22.7%)
BMI at baseline							
< 30 kg/m^2	5/134 (3.7%)	19/147 (12.9%)	9.2%	(2.9%, 15.5%)	30/146 (20.5%)	16.8%	(9.3%, 24.1%)
≥ 30 kg/m^2	8/81 (9.9%)	15/66 (22.7%)	12.8%	(0.8%, 24.9%)	13/56 (23.2%)	13.3%	0.5%, 26.2%)

Compiled by this reviewer from Table 14.4.1.1I.1- 14.4.1.1I-5, and 14.4.1.1M

As seen from the table above, 12-week CSBM overall responder rates were numerically higher for linaclotide subjects for regions, and BMI at baseline.

4.2.2 Study MCP-103-303

A summary of the results of the subgroup analyses of proportion of 12-week CSBM overall responders for Study MCP-103-303 is given below.

Subgroup Analyses of Proportion of 12-Wweek CSBM Overall Responders Study MCP-103-303

Subgroup	Placebo	Linaclotidde 133 ug	Difference (LIN133-PLA)	95% CI	Linaclotidde 266 ug	Difference (LIN266-PLA)	95% CI
Region							
Midwest	1/31 (3.2%)	9/30 (30.0%)	26.8%	(9.2%, 44.3%)	4/28 (14.3%)	11.1%	(-3.3%, 25.4%)
Northeast	0/18 (0.0%)	3/23 (13.0%)	13.0%	(-7.2%, 26.8%)	1/24 (4.2%)	4.2%	(-3.8%, 12.2%)
Southeast	6/105 (5.7%)	21/111 (18.9%)	13.2%	(4.7%, 21.7%)	25/109 (22.9%)	17.2%	(8.2%, 26.3%)
Southwest	0/27 (0.0%)	4/25 (16.0%)	16.0%	(1.6%, 30.4%)	4/23 (17.4%)	17.4%	(1.9%, 32.9%)
West	0/28 (0.0%)	9/28 (32.1%)	32.1%	(14.8%,9.4%)	8/32 (25.0%)	25.0%	(10.0%, 40.0%)
BMI at baseline							
< 30 kg/m^2	3/146 (2.1%)	33/158 (20.9%)	18.8%	(12.1%, 25.6%)	28/156 (17.9%)	15.8%	(9.4%, 22.3%)
≥ 30 kg/m^2	4/63 (6.3%)	13/59 (22.0%)	15.7%	(3.5%, 27.9%)	14/60 (23.3%)	17.0%	(4.7%, 29.3%)

Compiled by this reviewer from Table 14.4.1.1I.1- 14.4.1.1I-5, and 14.4.1.1M

As seen from the table above, 12-week CSBM overall responder rates were numerically higher for linaclotide subjects for regions, and BMI at baseline.

5. SUMMARY AND CONCLUSIONS

5.1 Statistical Issues and Collective Evidence

Study LIN-MD-01 showed that both linaclotide dose groups (133 µg and 266 µg) were statistically significantly better than placebo in terms of the primary efficacy endpoint, 12-week CSBM overall responder. The treatment differences were 9.9% and 14.7% for linaclotide 133 µg and 266 µg, respectively. Superiority was also shown for some secondary efficacy endpoints: change from baseline in 12-week CSBM frequency rate, change from baseline in 12-week SBM frequency rate, and change from baseline in 12-week stool consistency.

For the changes from baseline in CSBMs/week and SBMs/week, the treatment effects were slightly numerically greater for subjects in the 266 µg dose group than for those in the 133 µg dose group. For CSBM, the linaclotide treatment effects were 1.4 and 2.0 for the 133 µg and 266 µg dose groups, respectively, and the SBM effects were 2.3 and 2.6. The within-dose differences in these group effects (0.6 and 0.3) might not be considered clinical meaningful.

The efficacy results from Study LIN-MD-01 were replicated in Study MCP-103-303 for primary efficacy endpoint: 12-week CSBM overall responder. However, the treatment differences were 16.9% and 15.6% for linaclotide 133 µg and 266 µg, respectively.

This reviewer performed analyses of CSBM monthly responder by month. A monthly responder is a CSBM weekly responder for at least 3 of the 4 treatment period weeks for that month. A subject with missing response at a specific month was considered a non-responder for that month.

For Study LIN-MD-01, greater proportions of monthly responders in the linaclotide group were observed for each month of the study. The linaclotide 266 μg dose group showed numerically higher response rates than the linaclotide 133 μg group from Month 1 through Month 3. But, the dose group difference decreased to 5.7% by Month 3.

Contrary to the finding from Study LIN-MD-01, Study MCP-103-303 showed that the monthly responder rates for the linaclotide 266 μg was numerically lower than those for the linaclotide 133 μg for each month of the study. At Month 3, the linaclotide 266 μg was 6% less than that for linaclotide 133 μg.

To assess sustained efficacy, this reviewer performed post-hoc analyses using a more stringent definition of 12-week overall responder. A subject was considered to be a responder if the subject was a monthly responder for all 3 months. A subject was considered to be a monthly responder if the subject was a weekly responder for at least 3 out of 4 weeks in the month.

For this more stringent definition, both linaclotide doses (133 μg and 266 μg) were superior to the placebo in both studies. Treatment differences were 7.5% and 12.8%, for linaclotide 133 μg and 266 μg, respectively in Study LIN-MD-01. Similar treatment differences were observed in Study MCP-103-303 (10.0% and 9.1%).

To assess sustained efficacy, this reviewer also performed post-hoc analyses using another more stringent definition of overall responder. A subject was considered to be a responder if the subject was a 12-week overall responder and was a weekly responder for at least 3 of 4 weeks in Month 3.

For this responder definition, both linaclotide doses (133 μg and 266 μg) were superior to the placebo in both studies. Treatment differences were 9.9% and 12.0%, for linaclotide 133 μg and 266 μg, respectively in Study LIN-MD-01. Similar treatment differences were observed in Study MCP-103-303 (13.2% and 14.2%).

In conclusion, both studies (MCP-103-303 and LIN-MD-01) showed that both linaclotide doses (133 μg and 266 μg) were superior to the placebo for protocol-specified primary efficacy endpoint.

In review of safety, this reviewer found that greater proportions of subjects with adverse events in the linaclotide group compared with subjects in the placebo group for both studies (MCP-103-303 and LIN-MD-01). These included proportions of subjects with at least one treatment related AE (TRAE), withdrew due to AE, with at least one episode of diarrhea, or discontinued due to TRAE of diarrhea.

5.2 Conclusions and Recommendations

The sponsor has submitted two pivotal studies (MCP-103-303 and LIN-MD-01) to support the indication for treatment of chronic idiopathic constipation (CIC). A separate statistical review addresses the IBS-C indication.

Study LIN-MD-01 showed that both linaclotide dose groups (133 μg and 266 μg) were statistically significantly better than placebo in terms of the primary efficacy endpoint, overall CSBM responder (See Section 1.2.1. for definition of overall responder.) The treatment differences were 9.9% and 14.7% for the linaclotide 133 μg and 266 μg, respectively.

Superiority was also shown for some secondary efficacy endpoints: change from baseline in 12-week CSBM frequency rate, change from baseline in 12-week SBM frequency rate, and change from baseline in 12-week stool consistency.

The treatment effects for the CSBM and SBM frequency rates were numerically greater for the higher dose group, however, a clinically meaningful dose response difference might not be evident.

The efficacy results from Study LIN-MD-01 were replicated in Study MCP-103-303 for the primary efficacy endpoint: 12-week CSBM overall responder rate. The treatment differences were 16.9% and 15.6% for the linaclotide 133 μg and 266 μg treatment groups, respectively.

This reviewer performed post-hoc analyses using a more stringent definition of responder, requiring subjects to be monthly responders for all 3 months. A subject was considered to be a monthly responder if the subject was weekly responders for at least 3 weeks of 4 weeks in the month.

For this more stringent definition, both linaclotide doses were superior to the placebo in both studies. Treatment differences were 7.5% and 12.8%, for linaclotide 133 μg and 266 μg dose groups, respectively in Study LIN-MD-01. Similar treatment differences were observed in Study MCP-103-303 (10.0% and 9.1%).

In conclusion, both studies (MCP-103-303 and LIN-MD-01) showed that both linaclotide doses (133 μg and 266 μg) were superior to placebo for the protocol-specified primary efficacy endpoint.

Regarding safety, greater proportions of subjects with adverse events were observed in the linaclotide groups compared with the placebo group for both studies. Specifically, more linaclotide subjects had at least one treatment related AE (TRAE), withdrew due to AE, had at least one episode of diarrhea, or discontinued due to a TRAE of diarrhea.

6. APPENDIX

Table 1 Demographic and Baseline Characteristics – ITT Population Study LIN-MD-01

Characteristic	*Placebo (N = 215)*	*Linaclotide*		*Total (N = 633)*
		133 µg (N = 213)	*266 µg (N = 205)*	
Age, y, mean ± SD	47.0 ± 13.5	48.5 ± 12.3	47.3 ± 13.3	47.6 ± 13.0
≥ 65 y, %	27 (12.6)	24 (11.3)	22 (10.7)	73 (11.5)
Sex, n (%)				
Male	19 (8.8)	18 (8.5)	24 (11.7)	61 (9.6)
Female	196 (91.2)	195 (91.5)	181 (88.3)	572 (90.4)
Race, n (%)				
Caucasian	168 (78.1)	168 (78.9)	155 (75.6)	491 (77.6)
Non-Caucasian	47 (21.9)	45 (21.1)	50 (24.4)	142 (22.4)
Ethnicity, n (%)				
Hispanic	30 (14.0)	29 (13.6)	34 (16.6)	93 (14.7)
Non-Hispanic	185 (86.0)	184 (86.4)	171 (83.4)	540 (85.3)
Weight, kg, mean ± SD	77.2 ± 19.4	75.0 ± 15.5	74.5 ± 16.8	75.6 ± 17.4
Height, cm, mean ± SD	163.8 ± 7.9	164.2 ± 7.6	164.9 ± 8.6	164.3 ± 8.0
BMI. kg/m^2, mean ± SD	28.8 ± 7.2	27.8 ± 5.2	27.4 ± 5.7	28.0 ± 6.1

BMI = body mass index.
Data source: Table 14.2.1

Table 2 Efficacy Variables at Baseline – ITT Population Study LIN-MD-01

Parameter	*Placebo (N = 215)*	*Linaclotide*	
		133 µg/day (N = 213)	*266 µg/day (N = 202)*
	Mean ± SD		
Weekly CSBM rate	0.27 ± 0.52	0.26 ± 0.51	0.28 ± 0.55
Weekly SBM rate	1.82 ± 1.43	1.85 ± 1.50	1.94 ± 1.55
BSFS	2.32 ± 1.02	2.35 ± 1.05	2.34 ± 1.05
Straining score	3.24 ± 0.80	3.23 ± 0.91	3.32 ±0.84
Abdominal discomfort score	2.56 ± 0.84	2.47 ± 0.86	2.53 ± 0.91
Bloating score	2.82 ± 0.87	2.78 ± 0.84	2.74 ± 0.96
Constipation severity	3.31 ± 0.72	3.28 ± 0.74	3.34 ± 0.74

Baseline efficacy values were derived from the IVRS daily diary data collected in the pretreatment period, specifically the period from 14 days before randomization up to the time of randomization.

BSFS = Bristol Stool Form Scale; CSBM = complete spontaneous bowel movement; ITT = intent to treat; SBM = spontaneous bowel movement.

Data source: Table 14.2.4.

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Table 3 Subgroup Analyses of 12-Week CSBM Overall Responder- ITT Population Study LIN-MD-01

Subgroup Analyses of 12-Week CSBM Overall Responder
ITT Population
Study LIN-MD-01

Subgroup	Placebo	Linaclotidde 133 µg	Difference (LIN133-PLA)	95% CI	Linaclotidde 266 µg	Difference (LIN266-PLA)	95% CI
Gender							
Male	1/19 (5.3%)	3/18 (16.7%)	11.4%	(8.5%, 31.3%)	9/23 (39.1%)	33.8%	(11.5%, 56.2%)
Female	12/196 (6.1%)	31/195 (15.9%)	9.8%	(3.6%, 15.9%)	34/179 (19.0%)	12.9%	(6.2%, 19.5%)
Age							
<65	11/188 (5.9%)	27/189 (14.3%)	8.4%	(2.4%, 14.4%)	38/181 (21.0%)	15.1%	(8.3%, 22.0%)
≥65	2/27 (7.4%)	7/24 (29.2%)	21.8%	(1.1%, 42.4%)	5/21 (23.8%)	16.4%	(-4.3%, 37.1%)
Race							
White	8/168 (4.8%)	29/168 (17.3%)	12.5%	(5.9%, 19.1%)	38/152 (25.0%)	20.2%	(12.6%, 27.8%)
Black	5/42 (11.9%)	4/41 (9.8%)	-2.1%	(-15.5%, 11.2%)	4/46 (8.7%)	-3.2%	(-15.9%, 9.5%)
Other	0/5 (0.0%)	1/4 (25.0%)	25.0%	(-17.4%, 67.4%)	1/4 (25.0%)	25.0%	(-17.4%, 67.4%)
Region							
Canada	0/7 (0.0%)	0/5 (0.0%)	0.0%		1/7 (14.3%)	14.3%	(-11.6%, 40.2%)
Midwest	1/32 (3.1%)	5/25 (20.0%)	16.9%	(0.0%, 33.7%)	9/29 (31.0%)	26.9%	(10.0%, 45.8%)
Northeast	1/18 (5.6%)	2/13 (15.4%)	9.8%	(-12.5%, 32.1%)	4/11 (36.4%)	30.8%	(0.5%, 61.1%)
Southeast	8/98 (8.2%)	18/100 (18.0%)	9.8%	(0.6%, 19.1%)	19/96 (19.8%)	11.6%	(2.0%, 21.3%)
Southwest	1/26 (3.8%)	6/32 (18.8%)	15.0%	(-0.5%, 30.3%)	6/30 (20.0%)	16.2%	(0.0%, 32.3%)
West	2/34 (5.9%)	3/38 (7.9%)	2.0%	(-9.7%, 13.7%)	4/29 (13.8%)	7.9%	(-6.9%), 22.7%)
BMI at baseline							
< 30 kg/m^2	5/134 (3.7%)	19/147 (12.9%)	9.2%	(2.9%, 15.5%)	30/146 (20.5%)	16.8%	(9.3%, 24.1%)
≥ 30 kg/m^2	8/81 (9.9%)	15/66 (22.7%)	12.8%	(0.8%, 24.9%)	13/56 (23.2%)	13.3%	0.5%, 26.2%)

Compiled by this reviewer from Table 14.4.1.1I.1- 14.4.1.1I-5, and 14.4.1.1M

Table 4 Sensitivity Analyses of Secondary Endpoints– Study LIN-MD-01

Endpoint	Analysis	Mean change from baseline Placebo	Linaclotidde 133 µg	Linaclotidde 266 µg	LS Mean Difference (LIN133-PLA)	LS Mean Difference (LIN266-PLA)
CSBM	LOCF	0.633 (1.14)	2.079 (3.20)	2.662 (3.68)	1.490 (0.95, 2.03)	2.038 (1.49, 2.59)
	CC	0.703 (1.22)	2.099 (2.87)	2.651 (3.14)	1.471 (0.88, 2.06)	2.008 (1.41, 2.60)
	OC	0.641 (1.16)	2.102 (2.98)	2.715 (3.63)	1.496 (0.97, 2.02)	2.081 (1.55, 2.61)
	BOCF	0.585 (1.10)	1.713 (2.51)	2.205 (2.76)	1.156 (0.73, 1.58)	2.144 (1.20. 2.05)
	MI	0.658	2.090	2.618	1.469 (0.96, 1.98)	1.968 (1.45, 2.48)
SBM	LOCF	1.184 (2.07)	3.392 (3.77)	3.726 (4.41)	2.262 (1.60, 2.92)	2.553 (1.88, 3.22)
	CC	1.312 (1.78)	3.214 (3.24)	3.510 (3.94)	1.943 (1.23, 2.65)	2.256 (1.53, 2.98)
	OC	1.204 (2.05)	3.471 (3.60)	3.827 (4.35)	2.312 (1.67, 2.96)	2.632 (1.98, 3.29)
	BOCF	1.062 (1.65)	2.735 (2.92)	3.142 (3.57)	1.700 (1.17, 2.23)	2.087 (1.55, 2.62)
	MI	1.174	3.366	3.719	2.234 (1.60, 2.87)	2.556 (1.92, 3.19)
Stool Consistency	LOCF	0.580 (0.93)	1.728 (1.47)	1.908 (1.49)	1.169 (0.93, 1.41)	1.329 (1.09, 1.57)
	CC	0.563 (0.94)	1.766 (1.51)	1.959 (1.29)	1.160 (0.88, 1.44)	1.331 (1.05, 1.62)
	OC	0.569 (0.94)	1.752 (1.46)	1.956 (1.45)	1.198 (0.96, 1.43)	1.377 (1.14, 1.62)
	BOCF	0.481 (0.86)	1.402 (1.35)	1.655 (1.28)	0.940 (0.73, 1.15)	1.178 (0.96, 1.40)
	MI	0.580	1.737	1.918	1.714 (0.94, 1.41)	1.888 (1.11, 1.60)
Severity of Straining	LOCF	-0.504 (0.77)	-1.077 (0.92)	-1.198 (1.05)	-0.574 (-0.72, -0.43)	-0.637 (-0.79, -0.49)
	CC	-0.551 (0.75)	-1.167 (0.94)	-1.221 (0.94)	-0.544 (-0.72, -0.37)	-0.593 (-0.77, -0.41)
	OC	-0.512 (0.77)	-1.095 (0.91)	-1.219 (1.04)	-0.566 (-0.71, -0.42)	-0.651 (-0.80, -0.50)
	BOCF	-0.465 (0.69)	-0.922 (0.88)	-1.042 (0.92)	-0.467 (-0.60, -0.33)	-0.537 (-0.68, -0.40)
	MI	-0.537	-1.089	-1.251	-0.556 (-0.70. -0.41)	-0.651 (-0.80, -0.50)
Constipation Severity	LOCF	-0.322 (0.79)	-0.885 (0.97)	-0.971 (0.90)	-0.584 (-0.74, -0.43)	-0.631 (-0.79, -0.48)
	CC	-0.321 (0.73)	-0.909 (0.94)	-0.933 (0.87)	-0.619 (-0.80, -0.44)	-0.644 (-0.83, -0.46)
	OC	-0.329 (0.79)	-0.917 (0.96)	-0.998 (0.91)	-0.612 (-0.76, -0.46)	-0.649 (-0.80, -0.50)
	BOCF	-0.288 (0.65)	-0.725 (0.82)	-0.775 (0.76)	-0.452 (-0.58, -0.32)	-0.473 (-0.61, -0.34)
	MI	-0.347	-0.934	-0.977	-0.607 (-0.76, -0.45)	-0.612 (-0.76, -0.46)
Abdominal	LOCF	-0.292 (0.59)	-0.460 (0.68)	-0.513 (0.66)	-0.200 (-0.31, -0.09)	-0.228 (-0.34, -0.12)

Discomfort	CC	-0.288 (0.55)	-0.527 (0.68)	-0.537 (0.64)	-0.264 (-0.39, -0.14)	-0.277 (-0.40, -0.15)
	OC	-0.295 (0.59)	-0.465 (0.67)	-0.507 (0.67)	-0.201 (-0.31, -0.09)	-0.224 (-0.33, -0.12)
	BOCF	-0.271 (0.53)	-0.417 (0.63)	-0.460 (0.61)	-0.172 (-0.27, -0.07)	-0.192 (-0.29, -0.09)
	MI	-0.310	-0.492	-0.532	-0.215 (-0.32, -0.11)	-0.230 (-0.34, -0.12)
Bloating	LOCF	-0.235 (0.60)	-0.438 (0.73)	-0.474 (0.69)	-0.217 (-0.34, -0.10)	-0.261 (-0.38, -0.14)
	CC	-0.225 (0.57)	-0.411 (0.74)	-0.482 (0.67)	-0.241 (-0.38,-0.10)	-0.300 (-0.44, -0.16)
	OC	-0.235 (0.60)	-0.441 (0.72)	-0.474 (0.69)	-0.218 (-0.34, -0.10)	-0.265 (-0.38, -0.15)
	BOCF	-0.216 (0.54)	-0.375 (0.68)	-0.426 (0.63)	-0.170 (-0.28, -0.06)	-0.228 (-0.34, -0.12)
	MI	-0.249	-0.464	-0.490	-0.230 (-0.35, -0.11)	-0.265 (-0.39, -0.14)

Compiled from Tables 14.4.4.2.D-14.4.2.7.I

Table 5 Demographic and Baseline Characteristics – ITT Population Study MCP-103-303

Demographic Characteristic	Placebo (N=209)	133 ug (N=217)	266 ug (N=216)	All (N=642)
Age, years				
Mean (SD)	49.3 (14.3)	47.1 (14.2)	47.6 (14.2)	48.0 (14.3)
Median (Min, Max)	49.0 (18, 85)	47.0 (19, 82)	48.0 (18, 83)	48.0 (18, 85)
Age, n (%)				
18 to < 40 years	52 (24.9)	67 (30.9)	65 (30.1)	184 (28.7)
40 to < 65	129 (61.7)	123 (56.7)	124 (57.4)	376 (58.6)
≥ 65 years	28 (13.4)	27 (12.4)	27 (12.5)	82 (12.8)
Gender, n (%)				
Female	182 (87.1)	191 (88.0)	188 (87.0)	561 (87.4)
Male	27 (12.9)	26 (12.0)	28 (13.0)	81 (12.6)
Race, n (%)				
Asian	2 (1.0)	2 (0.9)	3 (1.4)	7 (1.1)
Black/African American	46 (22.0)	46 (21.2)	52 (24.1)	144 (22.4)
Caucasian	160 (76.6)	164 (75.6)	157 (72.7)	481 (74.9)
Other	1 (0.5)	5 (2.3)	4 (1.9)	10 (1.6)
Ethnicity, n (%)				
Hispanic/Latino	6 (2.9)	13 (6.0)	15 (6.9)	34 (5.3)
Not Hispanic/Lat.	203 (97.1)	204 (94.0)	201 (93.1)	608 (94.7)
Height, cm				
Mean (SD)	166.2 (8.6)	165.4 (8.4)	165.8 (8.5)	165.8 (8.5)
Median (Min, Max)	165.1 (142.2, 203.2)	165.1 (133.4, 190.5)	165.1 (137.2, 190.5)	165.1(133.4, 203.2)
Weight, kg				
Mean (SD)	77.0 (17.0)	76.5 (18.7)	76.9 (16.1)	76.8 (17.2)
Median (Min, Max)	76.7 (45.5, 155.0)	73.0 (47.9, 179.5)	75.4 (47.6, 158.8)	74.8 (45.5, 179.5)
BMI, kg/m^2				
Mean (SD)	27.8 (5.4)	27.9 (6.5)	28.0 (5.3)	27.9 (5.8)
Median (Min, Max)	27.6 (18.1, 50.4)	26.9 (15.1, 69.9)	27.4 (19.0, 48.6)	27.3 (15.1, 69.9)

Data Source: Section 14, Table 14.2.2
Age is calculated up to the informed consent date.
SD = Standard Deviation, BMI = Body mass index, defined as weight in kg divided by height in m^2.

Table 6 Efficacy Variables at Baseline – ITT Population Study MCP-103-303

Efficacy Parameter	Statistic	Placebo (N=209)	133 ug (N=217)	266 ug (N=216)	Total (N=642)
Weekly CSBM Rate	n	209	217	216	642
	Mean (SD)	0.3 (0.6)	0.3 (0.6)	0.2 (0.4)	0.3 (0.5)
	Median	0.0	0.0	0.0	0.0
	Min, Max	0.0, 2.4	0.0, 2.9	0.0, 2.0	0.0, 2.9
Weekly SBM Rate	n	209	217	216	642
	Mean (SD)	2.0 (1.6)	2.1 (1.6)	2.0 (1.6)	2.1 (1.6)
	Median	1.9	1.9	1.5	1.9
	Min, Max	0.0, 6.8	0.0, 6.8	0.0, 7.3	0.0, 7.3
Stool Consistency (BSFS)[a]	n	180	188	189	557
	Mean (SD)	2.4 (1.0)	2.4 (1.0)	2.5 (1.1)	2.4 (1.0)
	Median	2.3	2.4	2.5	2.3
	Min, Max	1.0, 6.0	1.0, 6.0	1.0, 6.0	1.0, 6.0
Straining[a]	n	180	188	189	557
	Mean (SD)	3.2 (0.9)	3.1 (0.8)	3.3 (0.9)	3.2 (0.9)
	Median	3.2	3.0	3.3	3.1
	Min, Max	1.0, 5.0	1.0, 5.0	1.0, 5.0	1.0, 5.0
Abdominal Discomfort	n	209	217	216	642
	Mean (SD)	2.5 (0.8)	2.5 (0.8)	2.5 (0.8)	2.5 (0.8)
	Median	2.5	2.5	2.6	2.5
	Min, Max	1.0, 4.3	1.0, 4.9	1.0, 4.7	1.0, 4.9
Bloating	n	209	217	216	642
	Mean (SD)	2.7 (0.9)	2.8 (0.9)	2.8 (0.9)	2.8 (0.9)
	Median	2.8	2.8	2.8	2.8
	Min, Max	1.0, 4.8	1.0, 5.0	1.0, 5.0	1.0, 5.0
Constipation Severity	n	209	217	216	642
	Mean (SD)	3.3 (0.7)	3.2 (0.8)	3.3 (0.7)	3.3 (0.8)
	Median	3.0	3.0	3.3	3.3
	Min, Max	1.3, 5.0	1.0, 5.0	1.0, 5.0	1.0, 5.0

Data Source: Section 14, Table 14.2.4
Baseline efficacy values are derived from the IVRS daily diary data collected in the Pretreatment Period, specifically the period of time from 14 days before randomization up to the time of randomization.
SD = Standard Deviation, Min = Minimum, and Max = Maximum.
[a] Patients who did not have an SBM at baseline had missing Stool Consistency and Straining baseline scores.

Table 7 Subgroup Analyses of 12-Week CSBM Overall Responder- ITT Population Study MCP-103-303

Subgroup Analyses of 12-Week CSBM Overall Responder
ITT Population
Study MCP-103-303

Subgroup	Placebo	Linaclotidde 133 µg	Difference (LIN133-PLA)	95% CI	Linaclotidde 266 µg	Difference (LIN266-PLA)	95% CI
Gender							
Male	1/27 (3.7%)	10/26 (38.5%)	34.8%	(14.5%, 54.7%)	6/28 (21.4%)	17.7%	(0.7%, 34.5%)
Female	6/182 (3.3%)	36/191 (18.8%)	15.5%	(9.4%, 21.7%)	36/188 (19.1%)	16.3%	(9.6%, 22.0%)
Age							
<65	6/181 (3.3%)	36/190 (18.9%)	15.6%	(9.5%, 21.8%)	37/189 (19.6%)	16.3%	(10.0%, 22.5%)
≥65	1/28 (3.6%)	10/27 (37.0%)	33.5%	(14.0%, 52.9%)	5/27 (18.5%)	16.4%	(-1.2%, 31.1%)
Race							
White	3/160 (1.9%)	37/164 (22.6%)	20.7%	(14.0%, 27.4%)	32/157 (20.4%)	18.5%	(11.9%, 25.2%)
Black	4/46 (8.7%)	9/46 (19.6%)	10.9%	(-7.2%, 26.8%)	8/52 (15.4%)	6.7%	(-6.1%, 19.4%)
Other	0/3 (0.0%)	0/7 (0.0%)	0.0%		2/7 (28.6%)	28.6%	(-4.9%, 62.0%)
Region							
Midwest	1/31 (3.2%)	9/30 (30.0%)	26.8%	(9.2%, 44.3%)	4/28 (14.3%)	11.1%	(-3.3%, 25.4%)
Northeast	0/18 (0.0%)	3/23 (13.0%)	13.0%	(-7.2%, 26.8%)	1/24 (4.2%)	4.2%	(-3.8%, 12.2%)
Southeast	6/105 (5.7%)	21/111 (18.9%)	13.2%	(4.7%, 21.7%)	25/109 (22.9%)	17.2%	(8.2%, 26.3%)
Southwest	0/27 (0.0%)	4/25 (16.0%)	16.0%	(1.6%, 30.4%)	4/23 (17.4%)	17.4%	(1.9%, 32.9%)
West	0/28 (0.0%)	9/28 (32.1%)	32.1%	(14.8%,9.4%)	8/32 (25.0%)	25.0%	(10.0%, 40.0%)
BMI at baseline							
< 30 kg/m^2	3/146 (2.1%)	33/158 (20.9%)	18.8%	(12.1%, 25.6%)	28/156 (17.9%)	15.8%	(9.4%, 22.3%)
≥ 30 kg/m^2	4/63 (6.3%)	13/59 (22.0%)	15.7%	(3.5%, 27.9%)	14/60 (23.3%)	17.0%	(4.7%, 29.3%)

Compiled by this reviewer from Table 14.4.1.1I.1- 14.4.1.1I-5, and 14.4.1.1M

Table 8 Sensitivity Analyses of Secondary Endpoints – Study MCP-103-303

Endpoint	Analysis	Mean change from baseline Placebo	Linaclotidde 133 µg	Linaclotidde 266 µg	LS Mean Difference (LIN133-PLA)	LS Mean Difference (LIN266-PLA)
CSBM	LOCF	0.617 (1.45)	2.077 (2.42)	2.212 (2.95)	1.470 (1.02, 1.92)	1.619 (1.17, 2.07)
	CC	0.529 (1.11)	2.213 (2.45)	2.459 (3.06)	1.701 (1.19, 2.21)	1.718 (1.19, 2.25)
	OC	0.582 (1.25)	2.095 (2.43)	2.251 (2.97)	1.520 (1.08, 1.96)	1.692 (1.25, 2.14)
	BOCF	0.529 (1.17)	1.920 (2.29)	1.946 (2.76)	1.399 (0.98, 1.81)	1.432 (1.02, 1.85)
	MI	0.597	2.103	2.213	1.514 (1.07, 1.95)	1.635 (1.19, 2.08)
SBM	LOCF	1.226 (2.14)	3.194 (3.13)	3.205 (3.66)	1.984 (1.40, 2.56)	1.978 (1.40, 2.56)
	CC	1.151 (1.86)	3.376 (3.05)	3.251 (3.56)	2.246 (1.61, 2.88)	2.084 (1.43, 2.74)
	OC	1.216 (1.94)	3.222 (3.07)	3.249 (3.61)	2.015 (1.45, 2.58)	2.632 (1.98, 3.29)
	BOCF	1.107 (1.84)	2.921 (2.95)	2.773 (3.30)	1.832 (1.31, 2.36)	1.663 (1.14, 2.19)
	MI	1.200	3.198	3.159	2.013 (1.45, 2.57)	1.957 (1.39, 2.52)
Stool Consistency	LOCF	0.581 (1.09)	1.834 (1.30)	1.761 (1.29)	1.247 (1.03, 1.47)	1.256 (1.04, 1.48)
	CC	0.555 (1.06)	1.824 (1.30)	1.763 (1.20)	1.241 (0.99, 1.49)	1.232 (0.98, 1.49)
	OC	0.579 (1.05)	1.837 (1.28)	1.773 (1.30)	1.247 (1.03, 1.46)	1.273 (1.06, 1.49)
	BOCF	0.517 (0.97)	1.599 (1.29)	1.429 (1.16)	1.083 (0.87, 1.29)	0.980 (0.77, 1.19)
	MI	0.588	1.830	1.766	1.249 (1.04, 1.46)	1.250 (1.03, 1.47)
Severity of Straining	LOCF	-0.485 (0.84)	-1.071 (0.84)	-1.194 (0.93)	-0.593 (-0.73, - 0.46)	-0.632 (-0.77, -0.50)
	CC	-0.504 (0.81)	-1.085 (0.83)	-1.207 (0.88)	-0.618 (-0.76, -0.47)	-0.650 (-0.80, -0.50)
	OC	-0.480 (0.83)	-1.088 (0.83)	-1.205 (0.93)	-0.613 (-0.74, -0.48)	-0.642 (-0.77, -0.51)
	BOCF	-0.453 (0.77)	-0.945 (0.81)	-1.002 (0.87)	-0.500 (-0.63, -0.37)	-0.482 (-0.61, -0.35)
	MI	-0.484	-1.066	-1.208	-0.596 (-0.73, -0.46))	-0.629 (-0.76, -0.49)
Constipation Severity	LOCF	-0.320 (0.75)	-0.884 (0.88)	-0.832 (0.89)	-0.610 (-0.75, -0.47)	-0.522 (-0.66, -0.38)
	CC	-0.301 (0.73)	-0.886 (0.89)	-0.870 (0.91)	-0.652 (-0.81, -0.49)	-0.573 (-0.74, -0.41)
	OC	-0.320 (0.74)	-0.905 (0.87)	-0.866 (0.88)	-0.623 (-0.76, -0.49)	-0.550 (-0.69, -0.41)
	BOCF	-0.288 (0.68)	-0.799 (0.83)	-0.757 (0.84)	-0.555 (-0.69, -0.42)	-0.479 (-0.61, -0.35)
	MI	-0.325	-0.903	-0.857	-0.625 (-0.77, -0.48)	-0.542 (-0.68, -0.40)
Abdominal Discomfort	LOCF	-0.321 (0.57)	-0.495 (0.56)	-0.458 (0.65)	-0.170 (-0.27, -0.07)	-0.116 (-0.21, -0.02)
	CC	-0.328 (0.59)	-0.468 (0.52)	-0.534 (0.65)	-0.150 (-0.26, -0.04)	-0.171 (-0.29, -0.06)

	OC	-0.307 (0.56)	-0.494 (0.55)	-0.465 (0.65)	-0.180 (-0.28, -0.08)	-0.137 (-0.23, -0.04)
	BOCF	-0.289 (0.54)	-0.443 (0.52)	-0.439 (0.62)	-0.150 (-0.24, -0.06)	-0.131 (-0.22, -0.04)
	MI	-0.322	-0.507	-0.480	-0.180 (-0.28, -0.08)	-0.136 (-0.23, -0.04)
Bloating	LOCF	-0.239 (0.57)	-0.479 (0.57)	-0.391 (0.68)	-0.239 (-0.34, -0.13)	-0.135 (-0.24, -0.03)
	CC	-0.224 (0.58)	-0.496 (0.56)	-0.466 (0.65)	-0.276 (-0.39, -0.16)	-0.200 (-0.32, -0.08)
	OC	-0.229 (0.56)	-0.480 (0.57)	-0.404 (0.68)	-0.248 (-0.35, -0.14)	-0.155 (-0.26, -0.05)
	BOCF	-0.216 (0.53)	-0.430 (0.54)	-0.370 (0.64)	-0.212 (-0.31, -0.11)	-0.137 (-0.24, -0.04)
	MI	-0.238	-0.485	-0.404	-0.246 (-0.35, -0.14)	-0.147 (-0.25, -0.04)

Compiled from Tables 14.4.4.2.1F-14.4.2.7.K

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/s/

MILTON C FAN
08/16/2012

MICHAEL E WELCH
08/16/2012
Concur with review.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDIES

IND/NDA Number:	IND 63-290/NDA 202-811
Drug Name:	Linaclotide Acetate
Indication(s):	104 Week Carcinogenicity in Rats and Mice
Applicant:	Sponsor: (b) (4) (b) (4)
Documents Reviewed:	Consult received on: July 23, 2011
Review Priority:	Priority
Biometrics Division:	Division of Biometrics -6
Statistical Reviewer:	Mohammad Nagem, Ph.D.
Concurring Reviewer:	Karl Lin, Ph.D.
Medical Division:	Division of Gastroenterology and Inborn Errors Products (DGIEP)
Reviewing Pharmacologist:	Niraj Mehta, Ph.D., David B. Joseph, Ph.D.
Project Manager:	Brian K. Strongin
Keywords:	Carcinogenicity, Dose response, Treatment of Irritable Bowel Syndrome with Constipation (c-IBS); Chronic Constipation and Other GI Disorders

Table of Contents

1. Background

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of Linaclotide to rats and mice when administered orally via oral gavage at appropriate drug levels for up to 105 weeks. Results of this review have been discussed with the reviewing pharmacologist Dr. Summan.

2. Rat Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and two control groups. Three hundred and fifty rats were randomly allocated to the three treated groups, and two control groups (control 1 and control 2). Each group has 70 rats per sex, the dose levels for treated groups were 300, 1000, or 3500 μg/kg/day. In this review these dose groups would be referred to as the low, medium and high dose groups, respectively. The two additional groups of 70 animals/sex/group served as the control and received the vehicle, 0.5% Methylcellulose (400 cps) in deionized water.

Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily throughout the duration of the study. Beginning Week 53, a third mortality check was added in the evening. Observations for clinical signs and masses were conducted on all main study (non-sentinel) animals weekly. Body weights were measured and recorded once during Week -1, once weekly for the first 13 weeks, and once every 4 weeks for the remainder of the study. Food consumption was measured and recorded once weekly for the first 13 weeks, and once every 4 weeks for the remainder of the study. Blood samples for clinical pathology evaluations (hematology parameters only) were collected from animals euthanized in extremis, if possible, and from all surviving animals at the scheduled terminal necropsy. Serological health screens were conducted on sentinel animals Pretest, and at 6, 12, 18, and 24 months. Necropsy examinations were performed on all main study animals dying spontaneously, euthanized in extremis, and at study termination, and selected tissues were microscopically examined.

2.1. Sponsor's analyses

2.1.1. Survival analysis

Survival function of each treatment group was estimated using the Kaplan-Meier product limit method. The dose response relationship[1] in mortality was tested using similar method as was suggested by Tarone. Pairwise comparisons of control and each treated group were performed using the Log-Rank test. All tests were conducted at two-tailed significance level of 0.05.

Sponsor's findings: Sponsor's analysis showed survival rates of 37%, 39%, 41% and 47% in combined controls, low, medium and high dose groups, respectively in male rats, and 37%, 26%, 36% and 27% respectively, in female rats groups. Sponsor concluded that there was no statistically significant treatment related effect on the survival in either sex groups, p-value= 0.5520 and p-value= 0.1019 for males and females

[1] In this review, the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor rate as dose increases.

respectively.

The survival curves of the two control groups for the male and female rats were compared using a pairwise comparison test. This test was not statistically significant for the male rats, p-value = 0.7268. However, the test was significant for female rats, p-value = 0.0160.

2.1.2. Tumor data analysis

The analysis for positive dose response relationship for tumor incidences among controls, low, medium and high dose groups and pairwise comparisons of controls and treated groups were performed using the methods outlined in the paper of Peto et al. (1982). For incidental tumors, the analysis intervals were: weeks 0 - 52, 53 - 78, 79 – 91, 92 – 104 and the terminal sacrifices. Exact permutation test were used for tumors with less than 10 incidences.

The analysis for dose response relationship was conducted at the significance levels of 0.005 (one tailed-level) for common tumors and 0.025 (one tailed-level) for rare tumors. Pairwise comparisons were conducted at the significance levels of 0.01 (one tailed-level) for common tumors and 0.05 (one tailed-level) for rare tumors. Common tumors were defined as those with a historical incidence in controls of 1% or higher and rare tumors as less than 1%.

The sponsor combined the two control groups as a comparator to the active treatment groups. In addition the sponsor compared both the control 1 and control 2 and concluded that there was no statistically significant difference in tumor incidences between these two controls.

Reviewer's comment: *The above significance levels for dose response relationship test were suggested by Lin and Rahman (1998) and those for pairwise comparisons were suggested by Haseman (1983) to adjust for multiple testing (to keep the false-positive rate at the nominal level of approximately 10%).*

Sponsor's findings: Sponsor's analyses showed no statistically significant positive dose response relationship or pairwise difference between control and any of the treated groups in any of the tested tumor types. The sponsor concluded that the incidence ranges of tumors in the treated groups fell within the reported historical control ranges, and the study, therefore, was interpreted as negative.

2.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically.

2.2.1. Survival analysis

The survival distributions of animals in all four treatment groups (combined control of control 1 and control 2 and 3 treatment groups) were estimated by the Kaplan-Meier product limit method. The dose response relationship was tested using the likelihood ratio test and homogeneity of survival distributions was tested using the log-rank test. The intercurrent mortality data are given in Tables 1A and 1B in the appendix for male and female rats, respectively. The Kaplan-Meier curves for survival rate are given in Figures 1A and 1B in the appendix for males

and females, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 2A and 2B in the appendix for males and females rats, respectively.

Reviewer's findings: The tests showed no statistically significant dose response relationships among treatment groups or differences between the combined vehicle control and any of the treated groups in survivals across treatment groups in both male and female rats.

2.2.2. Tumor data analysis

The tumor data were analyzed for dose response relationship and pairwise comparisons of combined vehicle control group with each of the treated groups were performed using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). One critical point for Poly-k test is the choice of the appropriate value of k. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of the tumor data of this study. For the calculation of p-values the exact permutation method was used. The tumor rates and the p-values of the tested tumor types using the combined control group are listed in Tables 3A and 3B in the appendix for males and females, respectively.

Multiple testing adjustment: The adjustment for the multiple dose response relationship testing was done using the criteria developed by Lin and Rahman (1998), which recommend the use of a significance level α=0.025 for rare tumors and α=0.005 for common tumors for a submission with two species, and the use of a significance level α=0.05 for rare tumors and α=0.01 for common tumors for a submission with only one species study in order to keep the false-positive rate at the nominal level of approximately 10%. A rare tumor is defined as one in which the published spontaneous tumor rate is less than 1%. The adjustment for multiple pairwise comparisons was done using the criteria developed by Haseman (1983), which recommend the use of a significance level α=0.05 for rare tumors and α=0.01 for common tumors, in order to keep the false-positive rate at the nominal level of approximately 10%.

As suggested by the reviewing pharmacologist Dr. Mehta, this reviewer did the analysis of the following tumor/organ combinations:

Male Rats:
All adenomas

Female Rats:
All adenomas

It should be noted that the recommended test levels by Lin and Rahman for the adjustment of multiple testing were originally based on the result of a simulation and an empirical study using the Peto method for dose response relationship analysis. However, some later simulation results by the same authors (Rahman and Lin, 2008) indicated similar usefulness of their recommendation for Poly-3 analysis also.

Reviewer's findings: Following tumor types showed p-values less than or equal to 0.05 either for dose response relationship and/or pairwise comparisons of control and treated groups.

Based on the criteria of adjustment for multiple testing of trends by Lin and Rahman there was statistically significant dose response relationship in the ADENOMA, INTERSTITIA in testes for the male rats.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons

Male Rats with Combined controls

Organ Name	Tumor Name	0 mg Cont N=140	300 mg Low N=70	1000 mg Med N=70	3500 mg High N=70	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
pituitary gland	ADENOMA, PARS DISTAL	74	39	40	50	0.0344	0.5212	0.4237	0.0574
skin	KERATOACANTHOMA, BEN	4	2	4	6	0.0499	0.6843	0.2863	0.1094
testes	ADENOMA, INTERSTITIA	0	1	0	4	**0.0049***	0.3516	.	0.0175

For the female rats, there was no statistically significant dose response relationship in the. However, based on the criteria by Haseman, the pair-wise comparisons of treated groups with the combined control, there was a statistically significant increase in tumor incidence of GRANULAR CELL TUMOR in uterus with cer. in the medium dose group when compared to the combined control for the female rats.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons
Female Rats with Combined controls

Organ Name	Tumor Name	0 mg Cont N=140	300 mg Low N=70	1000 mg Med N=70	3500 mg High N=70	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
ovaries	THECOMA, BENIGN	0	0	0	2	0.0358	.	.	0.0984
uterus with cer	GRANULAR CELL TUMOR,	1	1	4	2	0.1721	0.5126	**0.0482***	0.2415
vagina	GRANULAR CELL TUMOR,	2	2	2	5	0.0185	0.3490	0.4367	0.0347

3. Mouse Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and two control groups. Three hundred fifty mice of each sex were randomly allocated to the three treated and the two control groups. Each group had 70 mice in each of the sex groups. The treated groups received dose levels of 600, 2000, and 6000 µg/kg/day for males and females. The controls groups received the vehicle, 0.5% Methylcellulose in deionized water. In this review these dose groups would be referred to as the low, medium, high and "combined control" groups, respectively. Terminal sacrifice for the male and female mice occurred after 104 weeks of treatment (days 729-735).

Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals through Week 52, and three times daily for all surviving animals thereafter. Observations for clinical signs and masses were conducted on all main study (non-sentinel) animals weekly. Body weights and food consumption were measured and recorded for all main study animals weekly for the first 13 weeks, and every 4 weeks thereafter. Blood samples for evaluation of hematology parameters were collected from all main study animals euthanized in extremis, if possible, and from all surviving animals at the scheduled terminal necropsy. Serological health screens were conducted on sentinel animals pretest and at 6, 12, 18, and 24 months. Necropsy examinations were performed on all main study animals dying spontaneously, euthanized in extremis, and at study termination, and selected tissues were microscopically examined.

3.1. Sponsor's analyses

3.1.1. Survival analysis

Survival data from the mouse study were analyzed using the same statistical methodologies as those that were used to analyze the survival data from the rat study.

Sponsor's findings: The sponsor's analysis showed survival rates of 37%, 39%, 41% and 47%, in combined control, low, medium, and high dose groups, respectively, in males and 37%, 26%, 36% and 27%, respectively in females. Sponsor concluded that there was no statistically significant trend toward an increase in mortality in both sexes p-value= 0.2380 and p-value= 0.5504 for males and females, respectively. Pairwise comparison of the individual dosed group to the combined control was not statistically significant, all p-value based on the log-rank tests were >0.05 for both sexes. No relevant differences in survival between the control 1 and control 2 were found, p-value= 0.7880 and p-value= 0.4968, for males and females, respectively.

3.1.2. Tumor data analysis

Tumor data from the mouse study were also analyzed using the same statistical methodologies used to analyze the tumor data from the rat study.

Sponsor's findings: The sponsor's analysis showed no statistically significant positive dose response relationship in both sexes. Pairwise comparisons showed no statistically significant increased incidence of tumor in higher dose groups when compared to the combined vehicle control group, except in one tumor type. It was observed in the assessment of liver hepatocellular adenomas in high dose group in male mice in the comparison with the combined vehicle control.

There were no linaclotide-related neoplastic findings observed in treated males or females.
Neoplasms in this study were of the type typically seen in this strain and age of mouse. Differences in tumor incidence between control and linaclotide-treated animals were small
and should not be interpreted as biologically significant. There was no increased incidence of lung adenomas in males; instead, incidence was variable with the highest incidence occurring in control males (23.0, 18.6, 12.9, and 8.6 % in males at 0, 600, 2000, and 6000 μg/kg/day, respectively).

In females, the incidence of benign lung bronchiolar alveolar adenomas was 8.6 (pooled Control groups 1 and 2), 14.3, 15.7, and 21.4 % at 0, 600, 2000, and 6000 μg/kg/day, respectively. The incidence of lung adenomas in females at 6000 μg/kg/day compared to the controls combined was statistically significant (p-value of 0.0094) using Fisher's exact test (p-value <0.01). Statistically significant positive trends in the occurrence of female lung adenomas were also present (Cochran-Armitage trend test p-value of 0.0049, Peto trend test p-value of 0.0031). Benign lung bronchiolar alveolar adenomas are considered common tumors in CD-1 mice; therefore, p-values <0.005 for trend tests and <0.01 for pair-wise comparisons are statistically significant. The 21.4 % incidence of lung adenomas in females at 6000 μg/kg/day is comparable to the upper range of 20% from the historical control incidences of lung adenomas in female mice from 15 other studies (with a total of 21 control groups) conducted in this laboratory.

The incidences of lung benign bronchiolar alveolar adenomas in the two female control groups in this study were 10.0% and 7.1 %, which are lower than the average historical percentage of lung adenomas in female

control mice from other studies conducted at this laboratory. Additionally, there were no increases in lung bronchiolar alveolar hyperplasia (which is considered a pre-neoplastic finding) or malignant lung bronchiolar alveolar carcinoma in females that received linaclotide in this study, which would have been expected if there was any involvement of linaclotide in the induction of lung neoplasia in females. Based on the lower incidences in the two control groups compared with historical control incidences, the lack of linaclotide-related increases in bronchiolar alveolar hyperplasia or bronchiolar alveolar carcinomas, the occurrence in a single gender at the highest dose level, and the extremely common occurrence of this tumor type in mice of this strain, this finding of benign bronchiolar alveolar adenomas at 6000 μg/kg/day in female mice is not interpreted to represent a carcinogenic response related to the administration of linaclotide.

There were no other pair-wise or trend test p-values that were statistically significant according to the evaluation criteria for common and rare tumors. The pair-wise p-value of malignant multicentric hemangiosarcoma in females at 600 μg/kg/day was 0.0305, the pair-wise p-value of malignant uterine stromal sarcoma in females at 2000 μg/kg/day was 0.0346, and the pair-wise p-value of malignant lung bronchiolar alveolar carcinoma in males at 6000 μg/kg/day was 0.0402; all three of these tumors are considered common tumors in CD-1 mice; therefore, since their p-values were greater than 0.01 they were not statistically significant.

3.2. Reviewer's analyses

This reviewer independently performed survival and tumor data analyses of the mouse study. For the mouse data analyses this reviewer used similar methodologies as those he used to analyze the data from the rat study. Data used in this reviewer's analyses were provided by the sponsor electronically.

3.2.1. Survival analysis

The intercurrent mortality data are given in Tables 4A and 4B using combined control groups in the appendix for males and females, respectively. The Kaplan-Meier curves for death rate are given in Figures 2A and 2B in the appendix for males and females, respectively. Results for test of dose response relationship and homogeneity of survivals among treatment groups are given in Tables 5A and 5B in the appendix for males and females, respectively.

Reviewer's findings: The tests showed no statistically significant dose response relationship or differences between the controls and any of the treated groups in survivals across treatment groups in male or female mice.

3.2.2. Tumor data analysis

The tumor rates and the p-values of the tumor types tested for dose response relationship and pairwise comparisons of control and treated groups are given in Table 6A and 6B in the appendix for males and females, respectively.

As suggested by the reviewing pharmacologist Dr. Mehta, this reviewer did the analysis of the following tumor/organ combinations:

Male Mice:

1. All adenomas
2. All lymphomas

Female Mice:

1. All adenomas
2. All lymphomas

Reviewer's findings: Following tumor types showed p-values less than or equal to 0.05 either for dose response relationship or pairwise comparisons of control and treated groups.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons Female Mice with Combined controls

Organ Name	Tumor Name	0mg Cont N=140	600mg Low N=70	2000mg Med N=70	6000 mg High N=70	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male Mice									
lung	CARCINOMA, BRONCHIOL	11	8	6	12	0.0243	0.3885	0.5490	0.0378
Female Mice									
all	ADENOMA-All	27	18	15	22	0.0269	0.2039	0.3790	0.0272
	HAS-All	9	11	8	8	0.2074	0.0401	0.1531	0.1429
bone marrow, st	LYMPHOMA, MALIGNANT	3	6	4	5	0.1191	0.0459	0.1680	0.0777
liver	HEMANGIOSARCOMA, MAL	3	3	1	6	0.0199	0.3397	0.7984	0.0363
lung	ADENOMA, BRONCHIOLAR	12	10	11	15	0.0059	0.1683	0.0884	**0.0064***
lymph node, hep	LYMPHOMA, MALIGNANT	0	0	1	2	0.0296	.	0.3333	0.1066
multicentric ne	HEMANGIOSARCOMA, MAL	9	11	8	8	0.2074	0.0401	0.1531	0.1429
ovaries	HEMANGIOSARCOMA, MAL	0	3	0	0	0.7841	**0.0401***	.	.
pituitary gland	LYMPHOMA, MALIGNANT	0	3	0	0	0.7841	**0.0401***	.	.
uterus with cer	SARCOMA, STROMAL, MA	4	4	7	1	0.6946	0.2773	0.0317	0.8661

Based on the criteria of Lin and Rahman, the tumor incidences in the female mice of tumor incidences of ADENOMA, BRONCHIOL in Lung was statistically significant in the high dose when compared to the combined (pooled) control group. The tumor incidences of HEMANGIOSARCOMA MALIGNANT in ovaries and HEMANGIOSARCOMA MALIGNANT, BRONCHIOLAR in pituitary gland were also statistically significant in the low dose when compared to the combined (pooled) control group for the female mice.

4. Summary

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of Linaclotide in rats and mice when administered orally at appropriate drug levels for about 104 weeks.

Rat Study: Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and two control groups. Three hundred and fifty rats were randomly allocated to three treated groups, and the two control groups (control 1 and control 2). Each group has 70 rats per sex, the dose levels for treated groups were 300, 1000, or 3500 μg/kg/day. In this review these dosed groups would be referred to as the low, medium and high dose group, respectively. The two additional groups of 70 animals/sex/group served as the control and received the vehicle, 0.5% Methylcellulose (400 cps) in deionized.

The tests showed no statistically significant dose response relationship or differences in survival between the vehicle control and any of the treated groups in survivals across treatment groups in both male and female rats.

Based on the criteria of adjustment for multiple testing of trends by Lin and Rahman there was statistically significant dose response relationship in the ADENOMA, INTERSTITIA in testes for the male rats.

For the female rats, there was no statistically significant dose response relationship in the. However, based on the criteria by Haseman, the pair-wise comparisons of treated groups with the combined control, there was a statistically significant increase in tumor incidence of GRANULAR CELL TUMOR in uterus with cer. in the medium dose group when compared to the combined control for the female rats.

Mouse Study: Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and two control groups. Three hundred fifty mice of each sex were randomly allocated to the three treated groups and the two control groups. Each group has 70 mice in each of the sex groups. The treated groups received dose levels of 600, 2000, and 6000 μg/kg/day for the males and the females. The controls groups received the vehicle, 0.5% Methylcellulose in deionized water. In this review these dosed groups would be referred to as **the low, medium, high and "combined control" dose** group, respectively. Terminal sacrifice for the male and female mice occurred after 104 weeks of treatment.

The tumor incidences of CARCINOMA, BRONCHIOL in Lung was statistically significant in the high dose when compared to the combined (pooled) control group for the male mice. For the female mice the tumor incidences of ADENOMA, BRONCHIOL in Lung was also statistically significant in the high dose when compared to the combined (pooled) control group. The tumor incidences of HEMANGIOSARCOMA MALIGNANT in ovaries and HEMANGIOSARCOMA MALIGNANT, BRONCHIOLAR in pituitary gland were also statistically significant in the low dose when compared to the combined (pooled) control group for the female mice.

Mohammad Nagem, Ph.D.
Mathematical Statistician

Concur: Karl Lin, Ph.D.
Team Leader, Biometrics-6

cc:
Archival IND 63-290/NDA 202-811
Dr. Mehta
Dr. Joseph
Mr. Strongin
Dr. Machado
Dr. Lin
Ms. Lillian Patrician

5. Appendix

Table 1A: Intercurrent Mortality Rate Male Rats with Combined control

	Vehicle		LOW		MEDIUM		High	
	0 mg|kg|day		300 mg|kg|day		1000 mg|kg|day		3500 mg|kg|day	
	No. of		No. of		No. of		No. of	
Week	Death	Cum. %	Death	Cum. %	Death	Cum. %	Death	Cum. %
0 - 52	2	1.43	1	1.43	.	.	.	.
53 - 78	16	12.86	6	10.00	10	14.29	6	8.57
79 - 91	25	30.71	9	22.86	10	28.57	12	25.71
92 - 104	24	47.86	19	50.00	11	44.29	12	42.86
Ter. Sac.	45	32.14	23	32.86	27	38.57	31	44.29

Table 1B: Intercurrent Mortality Rate Female Rats with Combined control

	Vehicle		LOW		MEDIUM		High	
	0 mg|kg|day		300 mg|kg|day		1000 mg|kg|day		3500 mg|kg|day	
	No. of		No. of		No. of		No. of	
Week	Death	Cum. %	Death	Cum. %	Death	Cum. %	Death	Cum. %
0 - 52	.	.	3	4.29	1	1.43	2	2.86
53 - 78	21	15.00	17	28.57	13	20.00	14	22.86
79 - 91	26	33.57	17	52.86	15	41.43	13	41.43
92 - 104	31	55.71	12	70.00	16	64.29	17	65.71
Ter. Sac.	48	34.29	16	22.86	25	35.71	18	25.71

Table 2A: Tests for Dose Response Relationship and Homogeneity of Mortality Male Rats

P-value	
Test	**Combined control**
Dose Response	0.1597
Homogeneity	0.5264

Table 2B: Tests for Dose Response Relationship and Homogeneity of Mortality Female Rats

P-value	
Test	**Combined control**
Dose Response	0.1615
Homogeneity	0.0542

Table 3A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons
Male Rats with Combined control

Organ Name	Tumor Name	0 mg Cont N=140	300 mg Low N=70	1000 mg Med N=70	3500 mg High N=70	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
adrenal glands	ADENOMA, CORTICAL, B	5	2	1	3	0.4332	0.7764	0.9304	0.6270
	PHEOCHROMOCYTOMA, MA	12	9	3	3	0.9653	0.3118	0.9522	0.9627
all	ADENOMA-All	93	49	50	56	0.1122	0.6224	0.3741	0.1921
bone	OSTEOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
brain	ASTROCYTOMA, MALIGNA	1	2	0	1	0.5122	0.2823	1.0000	0.6064
	GRANULAR CELL TUMOR,	2	1	1	0	0.8497	0.7309	0.7309	1.0000
	MIXED GLIOMA, MALIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	OLIGODENDROGLIOMA, M	0	0	0	1	0.2242	.	.	0.3759
cavity, abdomin	CARCINOMA, SQUAMOUS	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	HEMANGIOSARCOMA, MAL	0	1	0	0	0.6278	0.3566	.	.
	SARCOMA, UNDIFFERENT	0	0	0	1	0.2207	.	.	0.3712
cavity, thoraci	CARCINOMA, SQUAMOUS	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	HIBERNOMA, MALIGNANT	1	1	2	0	0.7003	0.5878	0.2892	1.0000
ears	FIBROMA, BENIGN	0	1	0	0	0.6261	0.3516	.	.
heart	HEMANGIOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	SCHWANNOMA, BENIGN	0	0	1	1	0.1378	.	0.3516	0.3712
kidneys	LIPOSARCOMA, MALIGNA	0	0	0	1	0.2207	.	.	0.3712
liver	ADENOMA, HEPATOCELLU	5	3	3	3	0.4972	0.5804	0.5804	0.6270
	CARCINOMA, HEPATOCEL	2	1	0	0	0.9484	0.7337	1.0000	1.0000
	CHOLANGIOMA, BENIGN	0	1	0	0	0.6261	0.3516	.	.
	HEMANGIOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
lung	ADENOCARCINOMA, MALI	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	ADENOMA, BRONCHIOLAR	0	1	1	0	0.5164	0.3516	0.3516	.
lymph node, mes	HEMANGIOMA, BENIGN	0	0	1	0	0.4234	.	0.3516	.
	HEMANGIOSARCOMA, MAL	2	0	0	0	1.0000	1.0000	1.0000	1.0000
	LYMPHANGIOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
mammary gland	ADENOCARCINOMA, MALI	0	1	1	0	0.5164	0.3516	0.3516	.
	ADENOMA, BENIGN	0	0	0	1	0.2207	.	.	0.3712
nose, level c	ADENOMA, BENIGN	0	0	0	1	0.2207	.	.	0.3712
oral mucosa	CARCINOMA, SQUAMOUS	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	PAPILLOMA, SQUAMOUS	0	0	0	1	0.2207	.	.	0.3712
pancreas	ADENOMA, ACINAR CELL	0	0	0	1	0.2207	.	.	0.3712
	ADENOMA, ISLET CELL,	14	9	4	6	0.8174	0.4253	0.9351	0.8372
	CARCINOMA, ISLET CEL	10	2	1	6	0.3033	0.9664	0.9930	0.5951
parathyroid gla	ADENOMA, BENIGN	4	2	2	1	0.7827	0.6895	0.6895	0.9061
pituitary gland	ADENOMA, PARS DISTAL	74	39	40	50	0.0344	0.5212	0.4237	0.0574
	ADENOMA, PARS INTERM	1	1	1	0	0.7244	0.5878	0.5813	1.0000
preputial gland	CARCINOMA, SQUAMOUS	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LIPOSARCOMA, MALIGNA	0	1	0	0	0.6278	0.3566	.	.
prostate gland	ADENOCARCINOMA, MALI	0	0	1	0	0.4234	.	0.3516	.
	MYXOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
seminal vesicle	FIBROSARCOMA, MALIGN	0	0	1	0	0.4234	.	0.3516	.
skin	ADENOMA, BASAL CELL,	1	1	1	0	0.7244	0.5878	0.5813	1.0000
	CARCINOMA, BASAL CEL	0	1	0	0	0.6261	0.3516	.	.
	CARCINOMA, SQUAMOUS	0	1	0	0	0.6261	0.3516	.	.
	KERATOACANTHOMA, BEN	4	2	4	6	0.0499	0.6843	0.2863	0.1094
	PAPILLOMA, SQUAMOUS	1	0	0	1	0.3935	1.0000	1.0000	0.6064
skin, subcutis	FIBROMA, BENIGN	7	4	0	4	0.4974	0.5911	1.0000	0.6316
	FIBROSARCOMA, MALIGN	3	0	1	0	0.8902	1.0000	0.8217	1.0000
	HEMANGIOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LIPOSARCOMA, MALIGNA	0	1	0	0	0.6261	0.3516	.	.
	PILOMATRIXOMA, BENIG	1	0	0	0	1.0000	1.0000	1.0000	1.0000
small intestine	ADENOCARCINOMA, MALI	0	0	0	1	0.2242	.	.	0.3759
	FIBROMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000

spleen	FIBROSARCOMA, MALIGN	0	1	0	0	0.6261	0.3516	.	.
	HEMANGIOMA, BENIGN	0	1	0	0	0.6261	0.3516	.	.
	HEMANGIOSARCOMA, MAL	0	0	1	0	0.4234	.	0.3516	.
	LEIOMYOSARCOMA, MALI	1	0	0	0	1.0000	1.0000	1.0000	1.0000
stomach, nongla	PAPILLOMA, SQUAMOUS	0	0	0	1	0.2207	.	.	0.3712
tail	FIBROSARCOMA, MALIGN	0	0	0	1	0.2207	.	.	0.3712
	PAPILLOMA, SQUAMOUS	0	0	0	1	0.2207	.	.	0.3712
testes	ADENOMA, INTERSTITIA	0	1	0	4	0.0049*	0.3516	.	0.0175
	MESOTHELIOMA, BENIGN	0	0	0	1	0.2207	.	.	0.3712
thymus gland	THYMOMA, BENIGN	0	1	0	0	0.6261	0.3516	.	.
thyroid gland	ADENOMA, C-CELL, BEN	14	4	5	4	0.8751	0.9406	0.8675	0.9545
	ADENOMA, FOLLICULAR	3	1	2	2	0.3823	0.8331	0.5777	0.6150
	CARCINOMA, C-CELL, M	2	1	0	1	0.5899	0.7309	1.0000	0.7548
tongue	CARCINOMA, SQUAMOUS	1	0	0	0	1.0000	1.0000	1.0000	1.0000
zymbal`s gland	CARCINOMA, ZYMBALS G	1	0	1	1	0.3072	1.0000	0.5813	0.6064
zymbal`s gland	PAPILLOMA, BENIGN	0	1	0	0	0.6278	0.3566	.	.

Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons Female Rats with Combined control

		0 mg	300 mg	1000 mg	3500 mg	P_Value			
		Cont	Low	Med	High	Dos	P_Value	P_Value	P_Value
Organ Name	Tumor Name	N=140	N=70	N=70	N=70	Resp	C vs. L	C vs. M	C vs. H
adrenal glands	ADENOMA, CORTICAL, B	10	1	6	3	0.6148	0.9826	0.4946	0.8348
	CARCINOMA, CORTICAL,	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	PHEOCHROMOCYTOMA, MA	2	2	1	0	0.8436	0.3490	0.7222	1.0000
all	ADENOMA-All	108	53	59	55	0.2834	0.6868	0.7160	0.4040
brain	ASTROCYTOMA, MALIGNA	0	1	2	2	0.0648	0.3008	0.1207	0.1015
	EPENDYMOMA, BENIGN	0	0	1	0	0.4089	.	0.3451	.
cavity, abdomin	HIBERNOMA, MALIGNANT	0	0	1	0	0.4089	.	0.3451	.
cavity, thoraci	HIBERNOMA, MALIGNANT	3	2	0	2	0.3850	0.4810	1.0000	0.5122
clitoral glands	ADENOCARCINOMA, MALI	0	1	0	0	0.5867	0.3008	.	.
	ADENOMA, BENIGN	1	0	0	1	0.3522	1.0000	1.0000	0.5408
	CARCINOMA, SQUAMOUS	0	1	0	1	0.1877	0.3008	.	0.3162
head	SARCOMA, UNDIFFERENT	0	0	0	1	0.1947	.	.	0.3212
heart	SCHWANNOMA, BENIGN	2	0	1	0	0.7954	1.0000	0.7222	1.0000
liver	ADENOMA, HEPATOCELLU	1	1	0	2	0.1287	0.5126	1.0000	0.2349
	CARCINOMA, HEPATOCEL	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	CHOLANGIOMA, BENIGN	0	0	1	0	0.4115	.	0.3497	.
lymph node, mes	HEMANGIOMA, BENIGN	0	0	0	1	0.1911	.	.	0.3162
mammary gland	ADENOCARCINOMA, MALI	39	25	18	17	0.7374	0.1162	0.7545	0.6639
	ADENOLIPOMA, BENIGN	0	0	0	1	0.1947	.	.	0.3212
	ADENOMA, BENIGN	10	1	5	3	0.6357	0.9826	0.6417	0.8442
	FIBROADENOMA, BENIGN	64	24	34	22	0.9584	0.8529	0.6305	0.9794
	FIBROMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
nose, level b	CARCINOMA, MALIGNANT	0	0	0	1	0.1911	.	.	0.3162
oral mucosa	CARCINOMA, SQUAMOUS	0	0	0	1	0.1947	.	.	0.3212
ovaries	FIBROSARCOMA, MALIGN	0	0	0	1	0.1911	.	.	0.3162
	GRANULOSA CELL TUMOR	0	0	1	0	0.4115	.	0.3497	.
	THECOMA, BENIGN	0	0	0	2	0.0358	.	.	0.0984
pancreas	ADENOMA, ISLET CELL,	3	0	2	2	0.2490	1.0000	0.5736	0.5170
	CARCINOMA, ISLET CEL	3	0	0	0	1.0000	1.0000	1.0000	1.0000
parathyroid gla	ADENOMA, BENIGN	3	4	0	1	0.7816	0.1249	1.0000	0.7829
pituitary gland	ADENOMA, PARS DISTAL	96	48	56	52	0.0720	0.4986	0.2699	0.1141
	ADENOMA, PARS INTERM	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	CARCINOMA, PARS DIST	1	0	0	0	1.0000	1.0000	1.0000	1.0000
skin	ADENOMA, SEBACEOUS C	0	0	0	1	0.1911	.	.	0.3162
	CARCINOMA, SQUAMOUS	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	KERATOACANTHOMA, BEN	0	0	1	0	0.4115	.	0.3497	.
skin, subcutis	FIBROMA, BENIGN	2	1	0	0	0.9307	0.6615	1.0000	1.0000

	FIBROSARCOMA, MALIGN	0	2	0	0	0.6818	0.0920	.	.
	LIPOMA, BENIGN	0	1	0	0	0.5867	0.3008	.	.
	OSTEOSARCOMA, MALIGN	0	0	0	1	0.1947	.	.	0.3212
small intestine	FIBROMA, BENIGN	0	0	0	1	0.1911	.	.	0.3162
thymus gland	FIBROMA, BENIGN	0	0	0	1	0.1947	.	.	0.3212
thyroid gland	ADENOMA, C-CELL, BEN	15	3	8	7	0.3852	0.9486	0.5749	0.6041
	ADENOMA, FOLLICULAR	3	1	0	1	0.6440	0.7656	1.0000	0.7921
	CARCINOMA, C-CELL, M	1	0	1	1	0.2709	1.0000	0.5727	0.5340
urinary bladder	CARCINOMA, TRANSITIO	0	0	1	0	0.4089	.	0.3451	.
	GRANULAR CELL TUMOR,	0	0	0	1	0.1911	.	.	0.3162
uterus with cer	ADENOCARCINOMA, MALI	1	1	1	1	0.3294	0.5126	0.5727	0.5340
	ADENOMA, BENIGN	2	0	0	0	1.0000	1.0000	1.0000	1.0000
	CARCINOMA, SQUAMOUS	0	0	1	0	0.4115	.	0.3497	.
	FIBROMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	FIBROSARCOMA, MALIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	GRANULAR CELL TUMOR,	1	1	4	2	0.1721	0.5126	0.0482*	0.2415
	HEMANGIOSARCOMA, MAL	0	1	0	0	0.5867	0.3008	.	.
	LEIOMYOSARCOMA, MALI	1	1	0	0	0.8302	0.5126	1.0000	1.0000
	POLYP, STROMAL, BENI	10	3	4	3	0.7185	0.8079	0.7861	0.8393
	SARCOMA, STROMAL, MA	2	1	1	1	0.4901	0.6615	0.7281	0.6905
	SCHWANNOMA, MALIGNAN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
vagina	FIBROSARCOMA, MALIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	GRANULAR CELL TUMOR,	2	2	2	5	0.0185	0.3490	0.4367	0.0347
	LEIOMYOMA, BENIGN	0	0	0	1	0.1911	.	.	0.3162
zymbal's gland	ADENOMA, BENIGN	0	0	1	0	0.4089	.	0.3451	.
	CARCINOMA, ZYMBALS G	0	1	0	0	0.5867	0.3008	.	.
	PAPILLOMA, SQUAMOUS	1	0	0	0	1.0000	1.0000	1.0000	1.0000

Table 4A: Intercurrent Mortality Rate in Male Mice with Combined control

	Vehicle		LOW		MEDIUM		High	
	0 mg|kg|day		600 mg|kg|day		2000 mg|kg|day		6000 mg|kg|day	
	No. of		No. of		No. of		No. of	
Week	Death	Cum. %	Death	Cum. %	Death	Cum. %	Death	Cum. %
0 - 52	8	5.71	1	1.43	2	2.86	7	10.00
53 - 78	21	20.71	3	5.71	9	15.71	8	21.43
79 - 91	21	35.71	8	17.14	13	34.29	9	34.29
92 - 104	20	50.00	16	40.00	15	55.71	12	51.43
Ter. Sac.	69	49.29	42	60.00	31	44.29	34	48.57

Table 4B: Intercurrent Mortality Rate Female Mice with Combined control

	Vehicle		LOW		MEDIUM		High	
	0 mg|kg|day		2 mg|kg|day		10 mg|kg|day		20 mg|kg|day	
	No. of		No. of		No. of		No. of	
Week	Death	Cum. %	Death	Cum. %	Death	Cum. %	Death	Cum. %
0 - 52	11	7.91	4	5.71	3	4.29	6	8.57
53 - 78	21	23.02	9	18.57	22	35.71	10	22.86
79 - 91	18	35.97	14	38.57	5	42.86	10	37.14
92 - 104	39	64.03	13	57.14	6	51.43	21	67.14
Ter. Sac.	51	36.69	30	42.86	34	48.57	23	32.86

Table 5A: Tests for Dose Response Relationship and Homogeneity of Mortality Male Mice

	P-value
Test	**Combined control**
Dose Response	0.8184
Homogeneity	0.8168

Table 5B: Tests for Dose Response Relationship and Homogeneity of Mortality Female Mice

	P-value
Test	**Combined control**
Dose Response	0.4684
Homogeneity	0.5504

Table 6A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons Male Mice with Combined control

		0 mg	600 mg	2000 mg	6000 mg	P_Value			
		Cont	Low	Med	High	Dos	P_Value	P_Value	P_Value
Organ Name	Tumor Name	N=140	N=70	N=70	N=70	Resp	C vs. L	C vs. M	C vs. H
adrenal glands	ADENOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	ADENOMA, CORTICAL, B	1	1	0	0	0.8514	0.6041	1.0000	1.0000
	ADENOMA, SUBCAPSULAR	5	6	3	1	0.8745	0.1721	0.5501	0.9114
	CARCINOMA, SUBCAPSUL	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LYMPHOMA, MALIGNANT	3	2	2	2	0.3657	0.6144	0.5537	0.5370
	PHEOCHROMOCYTOMA, BE	1	0	0	0	1.0000	1.0000	1.0000	1.0000
all	ADENOMA-All	55	30	31	17	0.9852	0.6625	0.3758	0.9918
	HAS-All	12	5	7	3	0.8225	0.8257	0.5056	0.9259
	lymphoma-All	6	5	5	5	0.2196	0.3737	0.2996	0.2782
aorta	LYMPHOMA, MALIGNANT	1	1	0	0	0.8525	0.6089	1.0000	1.0000
bone marrow, fe	HEMANGIOSARCOMA, MAL	0	0	1	0	0.3889	.	0.3418	.
	LYMPHOMA, MALIGNANT	3	1	0	2	0.3344	0.8411	1.0000	0.5328
bone marrow, st	HEMANGIOSARCOMA, MAL	0	0	1	0	0.3889	.	0.3418	.
	LYMPHOMA, MALIGNANT	3	1	0	3	0.1428	0.8433	1.0000	0.3132
bone, femur	HEMANGIOSARCOMA, MAL	0	0	1	0	0.3889	.	0.3418	.
bone, sternum	CHONDROMA, BENIGN	0	0	0	1	0.1896	.	.	0.3290
	LYMPHOMA, MALIGNANT	1	0	0	2	0.0953	1.0000	1.0000	0.2550
brain	LYMPHOMA, MALIGNANT	0	1	0	1	0.1967	0.3697	.	0.3290
	MENINGIOMA, MALIGNAN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	OLIGODENDROGLIOMA, M	0	0	1	0	0.3889	.	0.3418	.
cavity, abdomin	HEMANGIOSARCOMA, MAL	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
cavity, thoraci	HEMANGIOSARCOMA, MAL	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
coagulating gla	LYMPHOMA, MALIGNANT	3	2	3	1	0.5941	0.6144	0.3301	0.7986
epididymides	HEMANGIOMA, BENIGN	0	1	0	0	0.6134	0.3697	.	.
	LYMPHOMA, MALIGNANT	4	2	3	0	0.8927	0.7200	0.4391	1.0000
esophagus	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
eyes	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
eyes, optic ner	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000

gallbladder	LYMPHOMA, MALIGNANT	2	1	1	1	0.4743	0.7541	0.7148	0.6979
harderian gland	ADENOMA, BENIGN	15	12	9	2	0.9851	0.2436	0.4285	0.9919
	LYMPHOMA, MALIGNANT	1	2	1	0	0.7326	0.3101	0.5653	1.0000
heart	LYMPHOMA, MALIGNANT	2	2	2	3	0.1200	0.4752	0.4187	0.2035
joint, tibiofem	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
kidneys	ADENOMA, TUBULAR CEL	0	0	1	1	0.1104	.	0.3376	0.3290
	LYMPHOMA, MALIGNANT	5	2	1	3	0.3479	0.8029	0.9195	0.5238
lacrimal glands	LYMPHOMA, MALIGNANT	3	2	1	2	0.3860	0.6144	0.8135	0.5370
large intestine	LYMPHOMA, MALIGNANT	0	0	1	0	0.3889	.	0.3418	.
		1	0	0	0	1.0000	1.0000	1.0000	1.0000
liver	ADENOMA, HEPATOCELLU	9	7	10	9	0.0618	0.3741	0.0603	0.0905
	CARCINOMA, HEPATOCEL	7	8	2	3	0.7529	0.1401	0.8729	0.7035
	HEMANGIOSARCOMA, MAL	7	5	5	2	0.7676	0.4795	0.3964	0.8616
	LYMPHOMA, MALIGNANT	5	2	3	5	0.0872	0.7995	0.5498	0.2021
lung	ADENOMA, BRONCHIOLAR	32	13	9	6	0.9965	0.9192	0.9828	0.9983
	CARCINOMA, BRONCHIOL	11	8	6	12	0.0243	0.3885	0.5490	0.0378
	HEMANGIOMA, BENIGN	0	1	0	0	0.6134	0.3697	.	.
	HEMANGIOSARCOMA, MAL	0	1	0	0	0.6134	0.3697	.	.
	LYMPHOMA, MALIGNANT	5	2	3	4	0.1818	0.8029	0.5550	0.3470
	MESOTHELIOMA, MALIGN	1	0	1	2	0.0848	1.0000	0.5653	0.2550
lymph node, axi	LYMPHOMA, MALIGNANT	0	1	0	0	0.6148	0.3735	.	.
lymph node, hep	LYMPHOMA, MALIGNANT	2	0	1	0	0.7718	1.0000	0.7148	1.0000
lymph node, ili	LYMPHOMA, MALIGNANT	1	1	1	0	0.6813	0.6061	0.5653	1.0000
lymph node, man	LYMPHOMA, MALIGNANT	3	2	1	3	0.1962	0.6144	0.8135	0.3217
lymph node, med	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
lymph node, mes	HEMANGIOMA, BENIGN	0	0	0	1	0.1896	.	.	0.3290
	HEMANGIOSARCOMA, MAL	2	1	0	0	0.9422	0.7496	1.0000	1.0000
	LYMPHOMA, MALIGNANT	4	4	2	3	0.3602	0.3324	0.6613	0.4194
lymph node, ren	LYMPHOMA, MALIGNANT	0	0	1	0	0.3889	.	0.3418	.
mammary gland	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
mesentery/perit	LYMPHOMA, MALIGNANT	1	0	0	1	0.3427	1.0000	1.0000	0.5484
multicentric ne	HEMANGIOSARCOMA, MAL	12	5	7	3	0.8225	0.8257	0.5056	0.9259
	LYMPHOMA, MALIGNANT	6	5	5	5	0.2196	0.3737	0.2996	0.2782
nerve, sciatic	LYMPHOMA, MALIGNANT	2	1	0	0	0.9429	0.7541	1.0000	1.0000
nose, level a	LYMPHOMA, MALIGNANT	2	0	0	1	0.4750	1.0000	1.0000	0.6979
nose, level b	LYMPHOMA, MALIGNANT	2	0	0	0	1.0000	1.0000	1.0000	1.0000
nose, level c	LYMPHOMA, MALIGNANT	2	0	0	0	1.0000	1.0000	1.0000	1.0000
nose, level d	LYMPHOMA, MALIGNANT	0	1	0	0	0.6134	0.3697	.	.
pancreas	CARCINOMA, ACINAR CE	0	1	0	0	0.6134	0.3697	.	.
	LYMPHOMA, MALIGNANT	3	2	1	1	0.6603	0.6144	0.8135	0.8038
peyers patch	LYMPHOMA, MALIGNANT	2	2	1	1	0.5369	0.4685	0.7148	0.6979
pituitary gland	ADENOMA, PARS DISTAL	1	0	2	0	0.5473	1.0000	0.2635	1.0000
	ADENOMA, PARS INTERM	0	0	0	1	0.1896	.	.	0.3290
preputial gland	LYMPHOMA, MALIGNANT	2	2	0	1	0.5874	0.4752	1.0000	0.6979
prostate gland	LYMPHOMA, MALIGNANT	1	2	2	0	0.7107	0.3101	0.2663	1.0000
salivary gland,	ADENOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LYMPHOMA, MALIGNANT	1	0	1	0	0.6274	1.0000	0.5682	1.0000
		3	0	0	0	1.0000	1.0000	1.0000	1.0000
			1	0	0	0.9783	0.8471	1.0000	1.0000
seminal vesicle	ADENOMA, BENIGN	0	0	0	1	0.1896	.	.	0.3290
	LYMPHOMA, MALIGNANT	4	1	1	1	0.6986	0.9035	0.8765	0.8640
skeletal muscle	LYMPHOMA, MALIGNANT	1	1	0	0	0.8508	0.6061	1.0000	1.0000
skin	LYMPHOMA, MALIGNANT	2	1	1	0	0.8145	0.7541	0.7148	1.0000
skin, subcutis	ADENOCARCINOMA, MALI	0	1	0	0	0.6134	0.3697	.	.
	FIBROUS HISTIOCYTOMA	0	0	1	0	0.3866	.	0.3376	.
	HEMANGIOSARCOMA, MAL	1	1	0	0	0.8496	0.6013	1.0000	1.0000
	LYMPHANGIOMA, BENIGN	0	0	1	0	0.3889	.	0.3418	.
	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	SARCOMA, UNDIFFERENT	2	0	0	0	1.0000	1.0000	1.0000	1.0000
	SCHWANNOMA, MALIGNAN	2	1	1	0	0.8106	0.7515	0.7065	1.0000
small intestine	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
				1	0	0.6257	1.0000	0.5653	1.0000

spinal cord, th	LYMPHOMA, MALIGNANT	0	0	1	0	0.3889	.	0.3418	.
spleen	HEMANGIOMA, BENIGN	0	1	0	0	0.6134	0.3697	.	.
	HEMANGIOSARCOMA, MAL	3	1	3	1	0.5341	0.8433	0.3385	0.7986
	LYMPHOMA, MALIGNANT	4	4	2	5	0.1039	0.3324	0.6613	0.1382
stomach, glandu	LYMPHOMA, MALIGNANT	2	2	1	1	0.5356	0.4752	0.7148	0.6979
stomach, nongla	LYMPHOMA, MALIGNANT	1	0	1	0	0.6257	1.0000	0.5653	1.0000
tail	HEMANGIOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
testes	ADENOMA, INTERSTITIA	3	4	5	0	0.8485	0.2292	0.0867	1.0000
	CARCINOMA, INTERSTIT	0	0	1	0	0.3866	.	0.3376	.
	HEMANGIOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
thymus gland	ADENOMA, FOLLICULAR	0	1	0	0	0.6134	0.3697	.	.
	LYMPHOMA, MALIGNANT	5	3	4	3	0.3777	0.6208	0.3575	0.5344
	THYMOMA, BENIGN	0	0	0	1	0.1926	.	.	0.3333
thyroid gland	ADENOMA, C-CELL, BEN	0	0	1	0	0.3866	.	0.3376	.
	ADENOMA, FOLLICULAR	1	1	0	0	0.8514	0.6041	1.0000	1.0000
	LYMPHOMA, MALIGNANT	2	1	0	1	0.5384	0.7541	1.0000	0.7037
tongue	LYMPHOMA, MALIGNANT	2	0	1	0	0.7718	1.0000	0.7148	1.0000
trachea	LYMPHOMA, MALIGNANT	0	0	0	1	0.1926	.	.	0.3333
ureters	LYMPHOMA, MALIGNANT	3	2	2	1	0.6124	0.6144	0.5537	0.7986
urinary bladder	LYMPHOMA, MALIGNANT	2	2	1	2	0.2788	0.4752	0.7148	0.4036
zymbal`s gland	LYMPHOMA, MALIGNANT	2	2	1	0	0.8407	0.4752	0.7148	1.0000

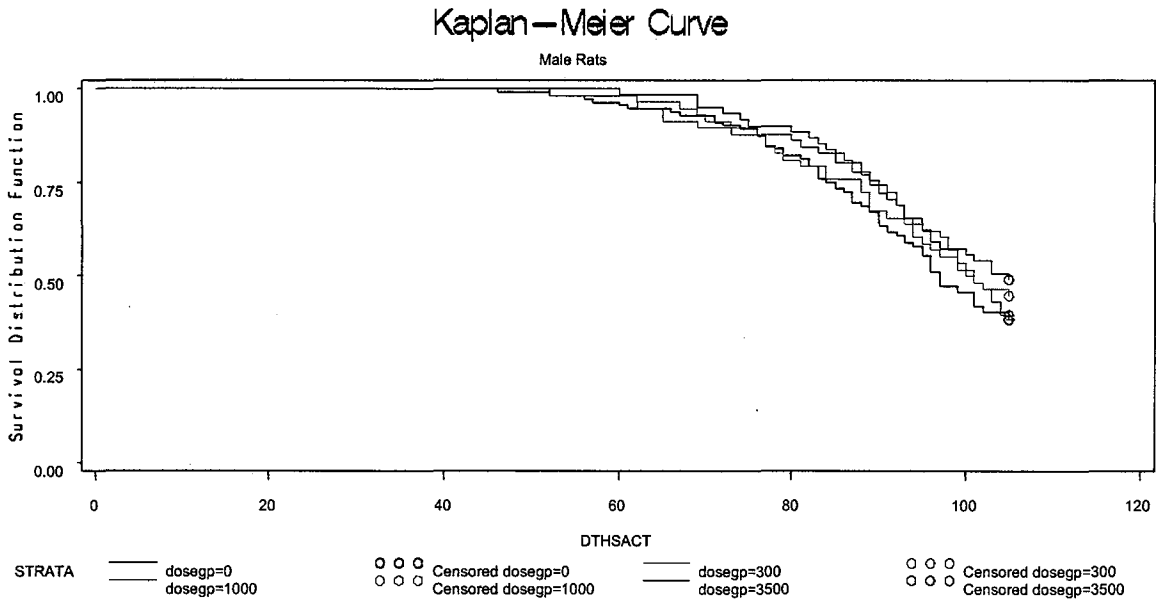
Table 6B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons
Female Mice with Combined control

Organ Name	Tumor Name	0 mg Cont N=139	600 mg Low N=70	2000 mg Med N=70	6000 mg High N=70	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
adrenal glands	ADENOMA, CORTICAL, B	0	1	0	0	0.6016	0.3467	.	.
	ADENOMA, SUBCAPSULAR	4	2	1	0	0.9473	0.6749	0.8661	1.0000
	LYMPHOMA, MALIGNANT	12	9	3	4	0.8457	0.2832	0.9209	0.8295
	PHEOCHROMOCYTOMA, BE	1	0	0	0	1.0000	1.0000	1.0000	1.0000
all	ADENOMA-All	27	18	15	22	0.0269	0.2039	0.3790	0.0272
	HAS-All	9	11	8	8	0.2074	0.0401	0.1531	0.1429
	LYM-all	22	14	9	14	0.2297	0.3466	0.7888	0.2386
aorta	LYMPHOMA, MALIGNANT	8	6	1	5	0.4146	0.3568	0.9740	0.4334
bone	OSTEOSARCOMA, MALIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
bone marrow, fe	HEMANGIOSARCOMA, MAL	1	0	0	2	0.0982	1.0000	1.0000	0.2487
	LYMPHOMA, MALIGNANT	3	5	3	5	0.0954	0.0913	0.3084	0.0777
bone marrow, st	LYMPHOMA, MALIGNANT	3	6	4	5	0.1191	0.0459	0.1680	0.0777
bone, femur	LYMPHOMA, MALIGNANT	1	1	1	0	0.6794	0.5717	0.5479	1.0000
	OSTEOSARCOMA, MALIGN	0	1	0	0	0.6016	0.3467	.	.
bone, sternum	HEMANGIOMA, BENIGN	0	0	0	1	0.1959	.	.	0.3288
	LYMPHOMA, MALIGNANT	4	5	2	4	0.2522	0.1588	0.6444	0.2446
bone, tibia	HEMANGIOSARCOMA, MAL	0	0	0	1	0.1959	.	.	0.3288
brain	GRANULAR CELL TUMOR,	0	1	0	0	0.6000	0.3423	.	.
	HEMANGIOSARCOMA, MAL	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LYMPHOMA, MALIGNANT	7	1	1	1	0.8888	0.9673	0.9616	0.9594
	MENINGIOMA, MALIGNAN	0	0	1	0	0.3943	.	0.3333	.
cavity, abdomin	HEMANGIOSARCOMA, MAL	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LIPOMA, BENIGN	0	0	0	1	0.1959	.	.	0.3288
cavity, thoraci	LYMPHOMA, MALIGNANT	1	1	0	0	0.8403	0.5717	1.0000	1.0000
	MESOTHELIOMA, MALIGN	1	1	0	0	0.8403	0.5717	1.0000	1.0000
clitoral glands	LYMPHOMA, MALIGNANT	5	1	2	3	0.2840	0.9231	0.7346	0.5118
esophagus	LYMPHOMA, MALIGNANT	2	0	1	1	0.4064	1.0000	0.6976	0.6976
eyes	LYMPHOMA, MALIGNANT	5	1	1	2	0.5248	0.9231	0.9095	0.7260
eyes, optic ner	LYMPHOMA, MALIGNANT	1	2	1	0	0.7344	0.2727	0.5541	1.0000
gallbladder	LYMPHOMA, MALIGNANT	3	2	2	1	0.6226	0.5632	0.5369	0.7984
harderian gland	ADENOCARCINOMA, MALI	0	1	0	1	0.1967	0.3423	.	0.3288
	ADENOMA, BENIGN	6	3	0	6	0.0836	0.6394	1.0000	0.1594
	LYMPHOMA, MALIGNANT	6	3	2	5	0.1653	0.6605	0.8050	0.2716
heart	LYMPHOMA, MALIGNANT	15	6	5	7	0.4727	0.8006	0.8516	0.6070
	MESOTHELIOMA, MALIGN	0	1	0	0	0.6016	0.3467	.	.
joint, tibiofem	LYMPHOMA, MALIGNANT	3	0	1	0	0.8641	1.0000	0.7957	1.0000
kidneys	LYMPHOMA, MALIGNANT	10	11	4	9	0.1826	0.0663	0.7450	0.1258
	RENAL MESENCHYMAL TU	0	1	0	0	0.6000	0.3423	.	.
lacrimal glands	LYMPHOMA, MALIGNANT	13	7	2	7	0.4348	0.5766	0.9819	0.4889
large intestine	LYMPHOMA, MALIGNANT	1	0	0	1	0.3528	1.0000	1.0000	0.5479
			1	0	2	0.1347	0.5717	1.0000	0.2487
		3	0	0	1	0.5997	1.0000	1.0000	0.7957
larynx	LYMPHOMA, MALIGNANT	1	1	1	0	0.6794	0.5717	0.5479	1.0000
liver	ADENOMA, HEPATOCELLU	2	1	1	2	0.2285	0.7185	0.7006	0.3992
	HEMANGIOSARCOMA, MAL	3	3	1	6	0.0199	0.3397	0.7984	0.0363
	LYMPHOMA, MALIGNANT	9	8	6	7	0.2307	0.2071	0.3852	0.2328
lung	ADENOMA, BRONCHIOLAR	12	10	11	15	0.0059	0.1683	0.0884	0.0064*
	CARCINOMA, BRONCHIOL	7	2	3	3	0.4844	0.8678	0.6929	0.6817
	LYMPHOMA, MALIGNANT	15	8	5	9	0.3052	0.5975	0.8661	0.3809
	MESOTHELIOMA, MALIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
lymph node, axi	LYMPHOMA, MALIGNANT	0	2	0	0	0.6732	0.1187	.	.
lymph node, hep	LYMPHOMA, MALIGNANT	0	0	1	2	0.0296	.	0.3333	0.1066
lymph node, ili	LYMPHOMA, MALIGNANT	4	1	0	3	0.2189	0.8831	1.0000	0.4133
lymph node, ing	LYMPHOMA, MALIGNANT	1	1	0	0	0.8423	0.5747	1.0000	1.0000
lymph node, man	LYMPHOMA, MALIGNANT	15	9	3	6	0.7386	0.4577	0.9697	0.7410

lymph node, med	LYMPHOMA, MALIGNANT	2	3	0	4	0.0684	0.2300	1.0000	0.0891
lymph node, mes	FIBROUS HISTIOCYTOMA	0	0	1	0	0.3918	.	0.3288	.
	HEMANGIOSARCOMA, MAL	0	1	2	0	0.5126	0.3423	0.1066	.
	LYMPHOMA, MALIGNANT	11	10	5	8	0.2898	0.1463	0.6718	0.2532
lymph node, ren	LYMPHOMA, MALIGNANT	3	1	0	3	0.1462	0.8142	1.0000	0.3037
mammary gland	ADENOACANTHOMA, MALI	2	0	0	0	1.0000	1.0000	1.0000	1.0000
	ADENOCARCINOMA, MALI	6	1	1	2	0.6221	0.9505	0.9401	0.8050
	ADENOMA, BENIGN	0	0	0	1	0.1959	.	.	0.3288
mammary gland	FIBROADENOMA, BENIGN	0	0	0	1	0.1959	.	.	0.3288
	LYMPHOMA, MALIGNANT	11	5	5	6	0.3758	0.7126	0.6720	0.5098
mesentery/perit	LYMPHOMA, MALIGNANT	5	3	2	2	0.6160	0.5621	0.7471	0.7302
multicentric ne	HEMANGIOSARCOMA, MAL	9	11	8	8	0.2074	0.0401	0.1531	0.1429
	LYMPHOMA, MALIGNANT	22	14	9	14	0.2297	0.3466	0.7888	0.2386
nerve, sciatic	LYMPHOMA, MALIGNANT	8	3	2	4	0.4304	0.7894	0.8961	0.5801
nose, level a	CARCINOMA, SQUAMOUS	0	0	1	0	0.3943	.	0.3333	.
	LYMPHOMA, MALIGNANT	1	1	1	0	0.6794	0.5717	0.5479	1.0000
nose, level b	LYMPHOMA, MALIGNANT	1	1	1	0	0.6794	0.5717	0.5479	1.0000
nose, level c	LYMPHOMA, MALIGNANT	2	1	1	1	0.4806	0.7211	0.6976	0.6976
	MENINGIOMA, BENIGN	0	0	1	0	0.3918	.	0.3288	.
nose, level d	LYMPHOMA, MALIGNANT	1	1	1	1	0.3289	0.5717	0.5479	0.5479
ovaries	ADENOMA, TUBULOSTROM	0	2	0	0	0.6732	0.1187	.	.
	CYSTADENOMA, BENIGN	5	4	3	1	0.8267	0.3801	0.5118	0.9095
	HEMANGIOSARCOMA, MAL	0	3	0	0	0.7841	0.0401*	.	.
	LYMPHOMA, MALIGNANT	10	9	5	9	0.1349	0.1773	0.6108	0.1258
	SEX-CORD/STROMAL TUM	1	1	2	0	0.6517	0.5689	0.2516	1.0000
oviducts	LYMPHOMA, MALIGNANT	1	1	0	0	0.8403	0.5717	1.0000	1.0000
pancreas	ADENOMA, ACINAR CELL	0	0	1	0	0.3943	.	0.3333	.
	ADENOMA, ISLET CELL,	0	0	1	0	0.3918	.	0.3288	.
	LYMPHOMA, MALIGNANT	8	9	5	5	0.4897	0.0843	0.4550	0.4264
peyers patch	LYMPHOMA, MALIGNANT	2	1	0	2	0.2333	0.7211	1.0000	0.3952
pharynx	LYMPHOMA, MALIGNANT	1	1	1	0	0.6794	0.5717	0.5479	1.0000
pituitary gland	ADENOMA, PARS DISTAL	6	3	2	4	0.2909	0.6501	0.7973	0.4153
	ADENOMA, PARS INTERM	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LYMPHOMA, MALIGNANT	0	3	0	0	0.7841	0.0401*	.	.
salivary gland,	LYMPHOMA, MALIGNANT	11	4	2	5	0.5161	0.8283	0.9636	0.6501
		12	7	2	5	0.6820	0.5076	0.9738	0.7050
		5	2	1	4	0.1943	0.7703	0.9113	0.3411
skeletal muscle	LYMPHOMA, MALIGNANT	1	0	0	0	1.0000	1.0000	1.0000	1.0000
		8	3	2	2	0.8070	0.7894	0.8961	0.8906
skin	LYMPHOMA, MALIGNANT	2	1	2	2	0.2096	0.7182	0.3994	0.3912
	PAPILLOMA, SQUAMOUS	1	0	0	0	1.0000	1.0000	1.0000	1.0000
skin, subcutis	FIBROSARCOMA, MALIGN	2	2	0	0	0.9212	0.4272	1.0000	1.0000
	LIPOSARCOMA, MALIGNA	0	0	1	0	0.3918	.	0.3288	.
	LYMPHOMA, MALIGNANT	3	1	2	0	0.8383	0.8191	0.5369	1.0000
	SARCOMA, UNDIFFERENT	0	0	1	0	0.3943	.	0.3333	.
small intestine	LYMPHOMA, MALIGNANT	0	0	0	1	0.1959	.	.	0.3288
			2	0	2	0.0974	0.1187	.	0.1066
		1	0	0	1	0.3528	1.0000	1.0000	0.5479
spinal cord, ce	LYMPHOMA, MALIGNANT	3	1	1	0	0.8943	0.8191	0.8038	1.0000
spinal cord, lu	LYMPHOMA, MALIGNANT	2	0	1	0	0.7778	1.0000	0.7037	1.0000
spinal cord, th	LYMPHOMA, MALIGNANT	2	1	1	0	0.8147	0.7211	0.7037	1.0000
spleen	HEMANGIOSARCOMA, MAL	2	1	1	3	0.0936	0.7155	0.6976	0.2031
	LYMPHOMA, MALIGNANT	15	12	5	9	0.4320	0.1821	0.8661	0.3809
stomach, glandu	LYMPHOMA, MALIGNANT	10	5	5	5	0.4610	0.6389	0.6108	0.5810
stomach, nongla	LYMPHOMA, MALIGNANT	1	1	0	1	0.3870	0.5747	1.0000	0.5510
	PAPILLOMA, SQUAMOUS	1	0	0	0	1.0000	1.0000	1.0000	1.0000
tail	HEMANGIOMA, BENIGN	0	0	1	0	0.3918	.	0.3288	.
	OSTEOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
thymus gland	LYMPHOMA, MALIGNANT	20	9	6	9	0.5827	0.7443	0.9293	0.6712
	THYMOMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
thyroid gland	ADENOMA, FOLLICULAR	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	LYMPHOMA, MALIGNANT	8	1	2	1	0.9019	0.9797	0.8988	0.9740

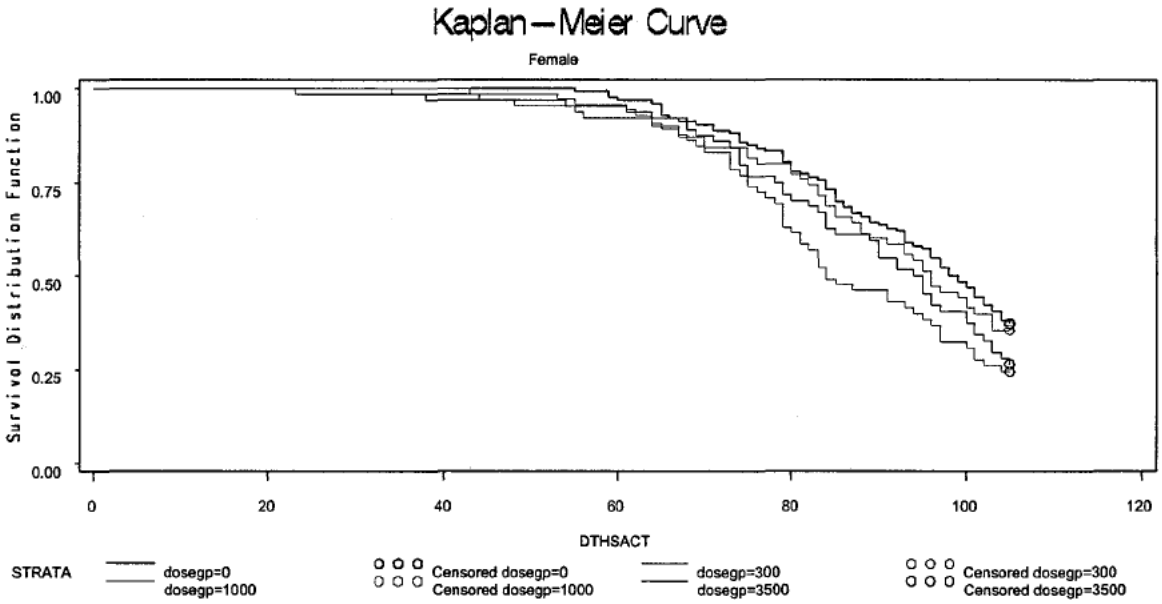
tongue	LYMPHOMA, MALIGNANT	10	4	2	4	0.6302	0.7833	0.9496	0.7391
	PAPILLOMA, SQUAMOUS	0	0	0	1	0.1959	.	.	0.3288
trachea	LYMPHOMA, MALIGNANT	2	1	1	1	0.4806	0.7211	0.6976	0.6976
ureters	LYMPHOMA, MALIGNANT	13	10	4	8	0.3798	0.2449	0.8761	0.3585
urinary bladder	LYMPHOMA, MALIGNANT	10	7	4	7	0.2604	0.3707	0.7330	0.2957
uterus with cer	ADENOCARCINOMA, MALI	4	2	0	1	0.7928	0.6710	1.0000	0.8684
	ADENOMA, BENIGN	0	0	1	0	0.3918	.	0.3288	.
	CARCINOMA, SQUAMOUS	0	0	2	0	0.3918	.	0.1096	.
	CHORIOCARCINOMA, MAL	0	0	0	1	0.1959	.	.	0.3288
	FIBROMA, BENIGN	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	GRANULAR CELL TUMOR,	0	0	0	1	0.1959	.	.	0.3288
	HEMANGIOSARCOMA, MAL	3	5	4	1	0.7263	0.0986	0.1715	0.7984
	LEIOMYOMA, BENIGN	4	0	2	2	0.3336	1.0000	0.6443	0.6443
	LEIOMYOSARCOMA, MALI	2	2	0	2	0.2955	0.4194	1.0000	0.3952
uterus with cer	LYMPHOMA, MALIGNANT	10	5	6	8	0.1126	0.6389	0.4609	0.1912
	POLYP, GLANDULAR, BE	2	0	1	1	0.4088	1.0000	0.7006	0.7006
	POLYP, STROMAL, BENI	16	4	5	2	0.9763	0.9576	0.8748	0.9940
	SARCOMA, STROMAL, MA	4	4	7	1	0.6946	0.2773	0.0317	0.8661
vagina	LYMPHOMA, MALIGNANT	7	3	1	4	0.3721	0.7293	0.9605	0.5040
	POLYP, BENIGN	0	0	0	1	0.1959	.	.	0.3288
zymbal's gland	CARCINOMA, ZYMBALS G	0	0	1	0	0.3943	.	0.3333	.
	LYMPHOMA, MALIGNANT	3	1	1	1	0.6079	0.8191	0.7984	0.7984

Figure 1A: Kaplan-Meier Survival Functions for Male Rats
Male Rats



X-Axis: Weeks, Y-Axis: Survival rates

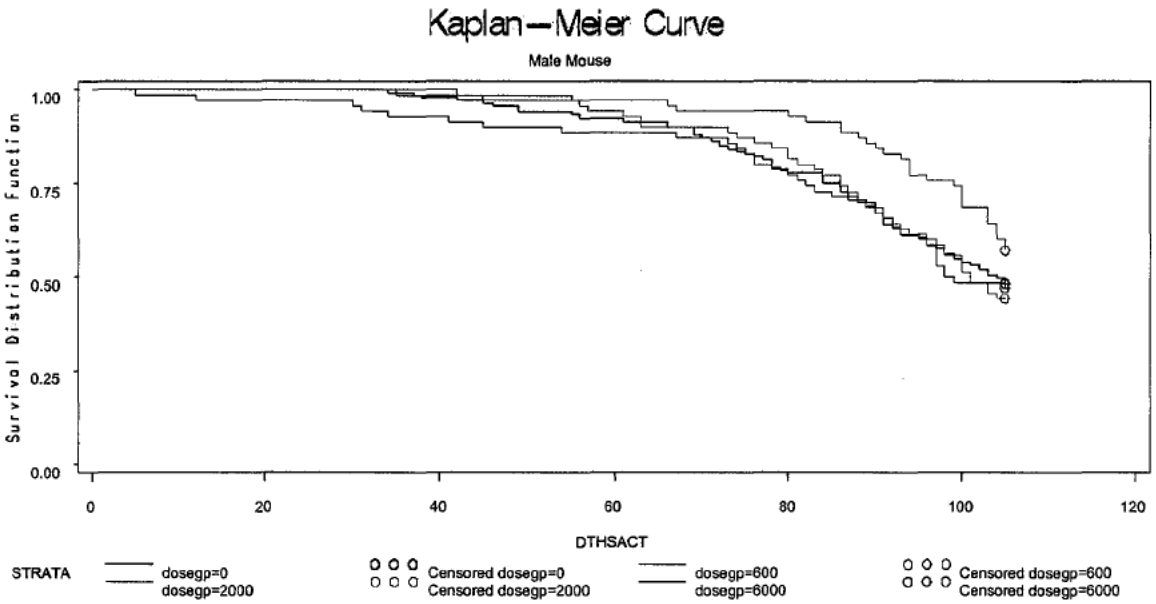
Figure 1B: Kaplan-Meier Survival Functions for Female Rats
Female Rats



X-Axis: Weeks, Y-Axis: Survival rates

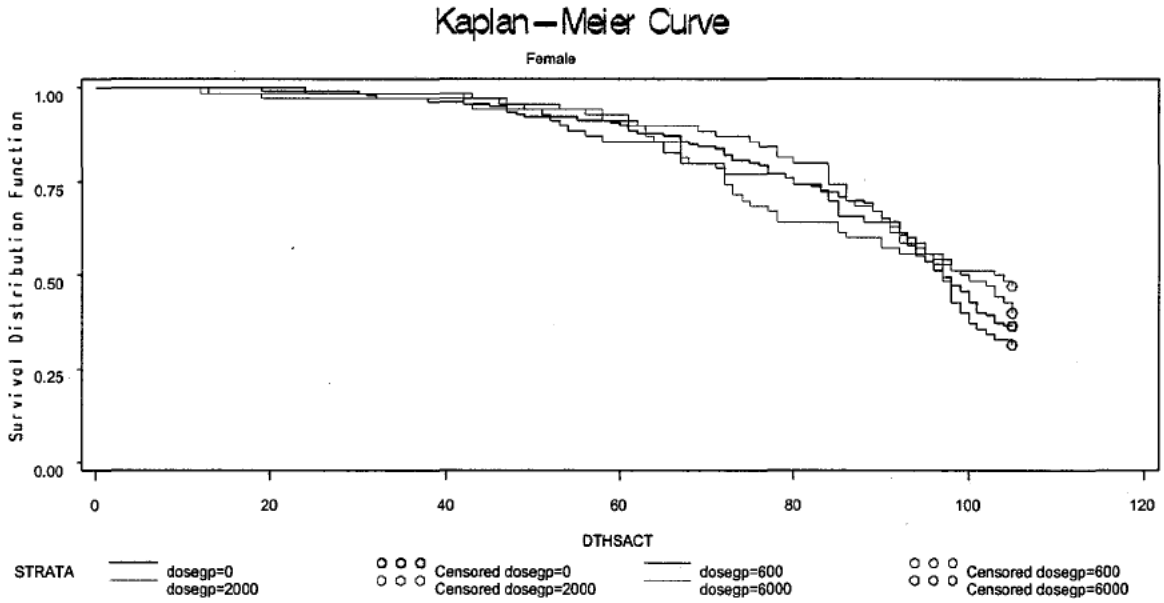
Figure 2A: Kaplan-Meier Survival Functions for Male Mice

Male Mice



X-Axis: Weeks, Y-Axis: Survival rates

Figure 2B: Kaplan-Meier Survival Functions for Female Mice
Female Mice



X-Axis: Weeks, Y-Axis: Survival rates

6. References:

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/s/

MOHAMED O NAGEM
02/06/2012

KARL K LIN
02/07/2012
Concur with review

STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

NDA/BLA Number:202-811 **Applicant:** Forest Laboratories, Inc. **Stamp Date:** 8/9/11

Drug Name: Linzess (linaclotide) **NDA/BLA Type:** Efficacy **Indication:** the treatment of IBS-C and chronic constipation

On **initial** overview of the NDA/BLA application for RTF:

	Content Parameter for RTF	Yes	No	NA	Comments
1A	Paper Submission: Index is sufficient to locate necessary reports, tables, data, etc.			**X**	Electronic submission
1B	Electronic Submission: Indexing and reference links within the electronic submission are sufficient to permit navigation through the submission, including access to reports, tables, data, etc.	X			
2	ISS, ISE, and complete study reports are available (including original protocols, subsequent amendments, etc.)	X			
3	Efficacy was investigated for gender, racial, and geriatric subgroups investigated.	X			Pooled studies
4	Data sets in EDR are accessible and conform to applicable guidances (e.g., existence of define.pdf file for data sets).	X			

IS THE STATISTICAL SECTION OF THE APPLICATION IS FILEABLE ? Yes

Content Parameter (possible review concerns for 74-day letter)	Yes	No	NA	Comment
Designs utilized are appropriate for the indications requested.	X			
Endpoints and methods of analysis are specified in the protocols/statistical analysis plans.	X			
Interim analyses (if present) were pre-specified in the protocol and appropriate adjustments in significance level made. DSMB meeting minutes and data are available.			X	No efficacy interim analysis planned.
Appropriate references for novel statistical methodology (if present) are included.		X		
Safety data organized to permit analyses across clinical trials in the NDA/BLA.	X			
Investigation of effect of dropouts on statistical analyses as described by applicant appears adequate.		X		No sensitivity analyses

Background

Ironwood Pharmaceuticals, Inc. submits this original NDA for linaclotide capsules, 145 µg and 290 µg, as an orally administered treatment for irritable bowel syndrome with constipation (IBS-C) and chronic constipation (CC), pursuant to the requirement of section 505(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 CFR 314 and supporting FDA guidelines. Ironwood and Forest are proposing LINZESS as the primary proprietary name.

Linaclotide, a 14-amino acid synthetic peptide, is a potent and selective guanylate cyclase-c (GC-C) receptor agonist structurally related to the endogenous guanylin peptide family. Activation of the GC-C receptor results in an increase in both intracellular and extracellular concentrations of cyclic guanosine monophosphate (cGMP). Elevation in intracellular cGMP stimulates secretion of chloride and bicarbonate into the intestinal lumen, through activation of the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel, resulting in increased intestinal fluid and accelerated transit. Extracellular cGMP decreases the activity of pain-sensing nerves, which is thought to be responsible for the observed reduction in visceral pain.

The sponsor has submitted two adequate and well-controlled studies (MCP-103-302) and LIN-MD-31) for the irritable bowel syndrome with constipation (IBS-C) indication and two adequate and well-controlled studies (MCP-103-303) and LIN-MD-01) for the chronic constipation (CC) indication.

This review will focus two studies (MCP-103-302 and LIN-MD-31) for irritable bowel syndrome.

All ADaM analysis datasets and study reports for this submission have been submitted in electronic Common Technical Document (eCTD) format to the EDR at: \\Cdsesub1\evsprod\NDA202811\0000.

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/s/

MILTON C FAN
10/06/2011

MICHAEL E WELCH
10/06/2011

STATISTICS FILING REVIEW

NDA Number: 202811 | **Applicant: Ironwood Pharmaceuticals, Inc.** | **Stamp Date: August 9, 2011**

Drug Name: Linaclotide capsules | **NDA Type: 505(b)(1) NDA** | **Indication: CC**

On **initial** overview of the Supplemental NDA application for RTF:

	Content Parameter for RTF	Yes	No	NA	Comments
1	Electronic Submission: Indexing and reference links within the electronic submission are sufficient to permit navigation through the submission, including access to reports, tables, data, etc.	X			
2	ISS, ISE, and complete study reports are available (including original protocols, subsequent amendments, etc.)	X			
3	Safety and efficacy were investigated for gender, racial, and geriatric subgroups investigated.	X			
4	Data sets in EDR are accessible and conform to applicable guidances (e.g., existence of define.pdf file for data sets).	X			

THE STATISTICAL SECTION OF THE APPLICATION IS FILEABLE

Content Parameter (possible review concerns for 74-day letter)	Yes	No	NA	Comment
Designs utilized are appropriate for the indications requested.	X			
Endpoints and methods of analysis are specified in the protocols/statistical analysis plans.	X			
Interim analyses (if present) were pre-specified in the protocol and appropriate adjustments in significance level made. DSMB meeting minutes and data are available.			X	Not present
Appropriate references for novel statistical methodology (if present) are included.			X	Not present
Safety data organized to permit analyses across clinical trials in the NDA/BLA.	X			
Investigation of effect of dropouts on statistical analyses as described by applicant appears adequate.	X			

STATISTICS FILING REVIEW

Background

Ironwood Pharmaceuticals, Inc. submits this original NDA for linaclotide capsules, 145 µg and 290 µg, as an orally administered treatment for irritable bowel syndrome with constipation (IBS-C) and chronic constipation (CC), pursuant to the requirement of section 505(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 CFR 314 and supporting FDA guidelines. Ironwood and Forest are proposing LINZESS as the primary proprietary name.

Linaclotide, a 14-amino acid synthetic peptide, is a potent and selective guanylate cyclase-c (GC-C) receptor agonist structurally related to the endogenous guanylin peptide family. Activation of the GC-C receptor results in an increase in both intracellular and extracellular concentrations of cyclic guanosine monophosphate (cGMP). Elevation in intracellular cGMP stimulates secretion of chloride and bicarbonate into the intestinal lumen, through activation of the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel, resulting in increased intestinal fluid and accelerated transit. Extracellular cGMP decreases the activity of pain-sensing nerves, which is thought to be responsible for the observed reduction in visceral pain.

The pivotal study designs were discussed during the end of phase 2 (EOP2) meeting held on May 15, 2008. The FDA agreed with using Rome II instead of Rome III criteria in enrollment and the primary endpoint. However, the FDA objected the sponsor proposal of including a four-week Randomized Withdrawal Period in only one of the two phase 3 efficacy trials for each indication to meet the Agency's request to assess the rebound effect of linaclotide. The format and content of the NDA submission was discussed at the pre-NDA meeting held on March 22, 2011.

The study data in the CDISC-SDTM 3.1.2 format and the analysis datasets in the CDISC ADaM, as well as the study reports for this submission have been submitted in electronic Common Technical Document (eCTD) format to the EDR at: \\Cdsesub1\evsprod\NDA202811\0000.

Overview of studies

Clinical development of this new molecule entity (NME) product was conducted under IND 63,290 by Ironwood Pharmaceuticals, Inc. and Forest Laboratories, Inc. This NDA submission contains data from the clinical development program, which is comprised of eleven completed studies (three phase 1 studies, two phase 2a studies, two phase 2b studies, and four phase 3 studies) and two ongoing open-label long-term safety clinical studies, all conducted in North America. A reported total of 75 healthy subjects and 4370 patients with IBS-C and CC have received at least one dose of linaclotide by Oct. 11, 2010. Among the data included are results from four phase 3 randomized, placebo-controlled, double-blind safety and efficacy trials (two in patients with IBS-C and two in CC patients) as well as from two phase 2b dose-ranging studies (one in each population) that support the safety and efficacy of linaclotide in the treatment of IBS-C and CC. This reviewer will evaluate CC indication and Dr. Milton Fan will evaluate IBS-C indication.

The four phase 2 and four phase 3 studies are summarized in the table below:

Type of Study	Study ID	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects	Study Subjects	Duration of Treatment
Safety and PD	MCP-103-004	Evaluation of safety and PD of multiple doses of Lin	Phase 2a, R, DB, PC, PG	97, 290, 966 µg Lin, or PBO; QD; multiple oral dose (liquid solution)	42 (12 Lin 97 µg, 10 Lin 290 µg, 10 Lin 966 µg, 10 PBO)	Patients with CC	14 days
Safety, Efficacy, and Dose Response	MCP-103-201	Evaluation of dose-ranging safety, efficacy, and dose response of multiple doses of Lin	Phase 2b, R, DB, PC, DRF, PG	72, 145, 290, 579 µg Lin, or PBO; QD; multiple oral dose (capsule)	309 (59 Lin 72 µg, 56 Lin 145 µg, 62 Lin 290 µg, 63 Lin 579 µg, 69 PBO)	Patients with CC	28 days
Efficacy and Safety	MCP-103-303	Evaluation of efficacy and safety of multiple doses of Lin	Phase 3, R, DB, PC, PG	145, 290 µg Lin, or PBO; QD; multiple oral dose (capsule) with RW	643 (217 Lin 145 µg, 217 Lin 290 µg, 209 PBO)	Patients with CC	16 weeks (12 weeks DB + 4 weeks RW)
Efficacy and Safety	LIN-MD-01	Evaluation of efficacy and safety of multiple doses of Lin	Phase 3, R, DB, PC, PG	145, 290 µg Lin, or PBO; QD; multiple oral dose (capsule)	633 (213 Lin 145 µg, 205 Lin 290 µg, 215 PBO)	Patients with CC	12 weeks
PD	MCP-103-005	Evaluation of dose-ranging PD of multiple doses of Lin	Phase 2a, R, DB, PC, PG	97, 966 µg Lin, or PBO; QD; multiple oral dose (liquid solution)	36 (12 Lin 97 µg, 12 Lin 966 µg, 12 PBO)	Patients with IBS-C	5 days
Safety, Efficacy, and Dose Response	MCP-103-202	Evaluation of dose-ranging safety, efficacy, and dose response of multiple doses of Lin	Phase 2b, R, DB, PC, DRF, PG	72, 145, 290, 579 µg Lin, or PBO; QD; multiple oral dose (capsule)	420 (79 Lin 72 µg, 82 Lin 145 µg, 85 Lin 290 µg, 89 Lin 579 µg, 85 PBO)	Patients with IBS-C	12 weeks
Efficacy and Safety	MCP-103-302	Evaluation of efficacy and safety of multiple doses of Lin	Phase 3, R, DB, PC, PG	290 µg Lin or PBO; QD; multiple oral dose (capsule)	805 (402 Lin 290 µg, 403 PBO)	Patients with IBS-C	26 weeks
Efficacy and Safety	LIN-MD-31	Evaluation of efficacy and safety of multiple doses of Lin	Phase 3, R, DB, PC, PG	290 µg Lin or PBO; QD; multiple oral dose (capsule) with RW	802 (406 Lin 290 µg, 396 PBO)	Patients with IBS-C	16 weeks (12 weeks DB + 4 weeks RW)

CC = chronic constipation; DB = double-blind; DRF = dose-range-finding; IBS-C = irritable bowel syndrome with constipation; Lin = linaclotide; PBO = placebo; PC = placebo-controlled; PD = pharmacodynamics; PG = parallel-group; QD = once daily; R = randomized; RW = randomized withdrawal;

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/s/

FREDA COONER
10/05/2011

MICHAEL E WELCH
10/05/2011